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MICROMORPHOLOGY OF PREHISTORIC HUMAN BONE FROM THE MESOLITHIC SITE OF MOITA DO SEBASTIÃO, PORTUGAL

BY

ESTHER MAY PALMER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF ARTS

DEPARTMENT OF ANTHROPOLOGY

EDMONTON, ALBERTA

SPRING 1987

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled MICROMORPHOLOGY OF PREHISTORIC HUMAN BONE FROM THE MESOLITHIC SITE OF MOITA DO SEBASTIÃO, PORTUGAL

submitted by Esther May Palmer

in partial fulfilment of the requirements for the degree of Master of Arts.

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(Supervisor)

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ABSTRACT

Bone samples from human skeletal remains from Moita do Sebastião, a Mesolithic shell midden site in Portugal, were analyzed to assess age at death, health status, and the bone microsorphology of these ancient people. Bone cores measuring 9 mm in diameter were extracted from the anterior midshaft of 32 femora. A bone wafer, measuring one millimeter in thickness, was cut from each core and embedded in Ward's Bio-Plastic. Thin sections 80-100 um thick were prepared from these embedded bone wafers. Embedding was necessary in order to protect the bone and prevent fracturing and disintegration during thin section preparation. Grinding and polishing of unprotected and undecalcified material was too destructive for this fragile archaeological material. An ash-gray discoloration of the bone in many areas of the thin sections obscured the osteons and prevented osteon counts for age determination. Analysis of the thin sections for indications of health status showed a low level of osteoporosis, early bone loss for males and no evidence of cortical thinning in females. Scanning electron microscopy revealed the presence of unidentified bacteria in association with the bone. Fluorescence microscopy of the thin sections revealed a fluorescent pattern in the bone similar to the labeling formed by the antibiotic tetracycline.

Key Words: mesolithic - Portugal - human bone - thin section - osteons - age - health - bacteria - tetracycline

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The general osteological analysis of the Portugal material used in the present thesis was generously provided by Dr. D. Lubell and Dr. M. Jackes. This analysis included data on stature estimates , the sex of each individual, preliminary age estimates based on the degree of dental attrition and the Nordin Index. The Nordin Index and the Singh Index were obtained from analysis of the radiographs of the Moita femora made in the District Hospital at Santiago do Caçem, Portugal.

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CHAPTER I

INTRODUCTION

Sometime between 12000 BP and 5000 BP in most regions of the Old World human subsistence strategies began to shift away from a nomadic lifestyle based on hunting of wild animals and gathering of wild plant foods to the planting and harvesting of domesticated food products.

There are many unanswered questions associated with this transition. Why and when did it occur in any one region and what were the main driving forces behind the change? Was the transition rapid, as originally suggested by Childe (1936), or did it involve a gradual blending of old and new elements as suggested by Flannery (1965)? What was the influence of demographic pressures (Binford 1968)? It has been suggested that the shift from foraging to food production may have been accompanied by a decrease in the quality and quantity of nutrition and thus an increase in disturbances of bone and tooth formation (Nickens 1976; Cohen 1977). If this is so, an assessment of bone microstructure may reveal changes indicating nutritional stress.

A major question concerns the effect of the transition on health and longevity (Angel 1969). Were there benefits in terms of better health and longer life or did Neolithic populations pay for the change in subsistence strategy with inferior health and shorter life? What were the effects of the transition on the biology of ancient human populations? Are there discernible

skeletal changes indicating biological response to the change in subsistence?

This phase in prehistory is particularly suitable for researching possible long term consequences of the transition in terms of differences in human life-span, age at death, health and disease, nutrition and demography. A technique, in the developmental stages, proposes to test a method whereby archaeological skeletal samples may be compared and demographic trends identified (Jackes and Lubell nd). If comparable data can be obtained, demographic variables can be used to determine spatial and temporal continuity in both the Old and New Worlds. The incidence of specific skeletal traits or variations in growth patterns can be noted through time and compared between populations.

Skeletal responses to dietary change and the impact of such changes on general health may have been considerable, but have so far received only limited study and are thus incompletely known. A major research project was launched in 1984 to study these problems using data from sites spanning the Mesolithic-Neolithic transition in Portugal (Lubell et al. 1984). In-depth research, utilizing a multidisciplinary approach, will incorporate data from archaeological investigations (zooarchaeology, geoarchaeology, geomorphology, palaeobotany, palynology) with data from human osteology, palaeodemography and palaeopathology to examine the effects on human biology of prehistoric changes in diet, diet, technology, culture, and environment.

Portugal has a large number of Mesolithic and Neolithic

skeletal samples that can be analyzed. These samples represent a wide range of ecological conditions since they were excavated from sites in coastal, estuarine, and inland regions. Foremost among these are the Mesolithic shell middens at Muge in southwestern Portugal. While these sites have been the subject of considerable archaeological research (Roche 1972), the numerous human skeletal remains are virtually unstudied (Ferembach 1972; Newell et al. 1979). Because of their large skeletal collections the Muge middens provide an opportunity to test hypotheses generated by the numerous unanswered questions associated with the Mesolithic-Neolithic transition. Modern methods of assessing prehistoric diet, palaeodemography, and palaeopathology have had limited application in the study of human biology during the transition from hunting and gathering to food production in Portugal (Lubell et al. 1986a).

Archaeological samples of human bone can be analyzed for trace elements and stable isotopes which permits reconstructing the diet of prehistoric populations and detecting dietary shifts through time (Wing and Brown 1979). Also, possible associations between trace elements and stable isotopes with pathology (Huss-Ashmore et al. 1982), sex, and status can be examined (Blakely and Beck 1981; Lambert et al. 1982).

Stable isotope analysis has detected the prehistoric shift to maize cultivation in eastern North America (Vogel and Van der Merwe 1977; Van der Merwe and Vogel 1978; Bender et al. 1981), in South America (Van der Merwe et al. 1981) and in Mexico (DeNiro and Epstein 1981). Trace element analysis has also

indicated prehistoric dietary change to food cultivation in eastern North America (Gilbert 1975, cited by Gilbert 1985; Schoeninger and Peebles 1981; Lambert et al. 1982) and in the midwestern United States (Price and Kavanagh 1982).

These methods may offer a potential solution to the question of when agriculture began in any one region. Although by themselves they cannot provide a specific date for subsistence change they may, in combination with more precise dating methods, assist in providing a better indication of when the change actually occurred.

The recent anthropological interest in microscopic analysis of thin sections of human bone has been encouraged by the desire to extract as much information as possible from the skeletal remains recovered from archaeological sites. Much of our knowledge of the microstructural features in bone and the development of histological techniques has been provided by microscopists such as Havers (1692), Howship (1817), Goodsir and Goodsir (1845), Tomes and de Morgan (1853), and others in their studies of normal and pathological changes in bone (cited and reviewed by Hancox 1972). The fact that bone is a living tissue and has a discrete microanatomy that is often preserved in fossil bone has been known for many years. As early as 1849, Quekett identified the gross histological structures of bone in thin sections of animal fossils (cited by Graf 1949; Hancox 1972). The preservation of histological detail in ancient human bone has been noted in Neandertal bone (Ascenzi 1955), in Homo erectus material (Mallegni et al. 1983), and in other human fossil

populations (Graf 1949; Salomon and Haas 1967). These findings indicate the potential value of microscopic analysis for anthropological research into health conditions and age at death in ancient populations.

An early method developed in 1911 by Balthazard and Lebrun for estimating age (Deslypere and Baert 1958) involved measuring the diameter of Haversian canals viewed in thin sections, however, the technique was not satisfactory because the age estimates obtained with this method were too unreliable. The observation of changes in the histological structure of bone with age (Jowsey 1960; Currey 1964) prompted Kerley (1965) to formulate a method for estimating age from microscopic analysis of thin sections of cortical bone.

Four methods for estimating age from human bone were developed subsequent to the original attempt by Balthazard and Lebrun (Kerley 1965; Ahlqvist and Damsten 1969; Singh and Gunberg 1970; Thompson 1979). All of these later techniques utilize thin sections of cortical bone for micromorphological analysis. The techniques are similar in purpose but they use different combinations of the microstructural features, such as the number of intact osteons, fragments of osteons (partially resorbed osteons), circumferential bone (non-Haversian bone), Haversian canals (vascular canals), and primary vascular canals (non-Haversian canals) for quantification and measurement.

These techniques are based on the observation that microstructural changes occur progressively with increasing age. For instance, in long bones during the period of rapid bone

growth most of the diaphyseal cortex consists of circumferential lamellar bone. Remodeling of this bone begins at approximately four years of age in present day populations and continues throughout the lifespan of the individual (Jowsey 1960). The result of this activity is a decrease in the percentage of circumferential bone and non-Haversian canals and an increase in the number of osteons and fragments.

A technique for accurately estimating age at death from small portions of the human skeleton is important since archaeological bone or bone from a forensic context is often fragmentary and only the femora, which are often the better preserved portions, may be suitable for microscopic analysis. Methods that rely on the pubic symphysis for determining age (Todd 1920; McKern and Stewart 1957; Gilbert and McKern 1973) may be impossible to use because the pelves are missing, damaged or poorly preserved.

Palaeodemography and palaeopathology have both been studied with microscopic techniques. Histological methods for estimating age have been applied in the study of life expectancy in ancient societies to provide a demographic profile of ossuary populations (Ubelaker 1974; Pfeiffer 1985). Estimates of age at death in archaeological populations have been determined by microscopic assessment of thin sections of bone (Laughlin et al. 1979; Thompson and Gunness-Hey 1981; Thompson and Cowen 1984). It is essential to have estimates of age at death in order to assess the health of such societies (Weinstein et al. 1981).

Histological analysis at the bone tissue level is a more sensitive approach than macromorphological analysis in obtaining

information on the nutrition and health status of archaeological populations (Martin et al. 1985). Since the microstructural features of the skeleton are responsive to the influences of both the internal and external environment, nutritional deficiences or a change in subsistence can affect these structures (Acsadi and Nemeskeri 1970).

Microscopic analysis of bone can provide valuable information on the nutrition and health status of an individual. Alterations in the bone remodeling rate (resorption and formation by bone cells) may indicate nutritional stress, dietary change, or pathology (Stout and Simmons 1979; Richman et al. 1979; Martin et al. 1981; Huss-Ashmore et al. 1982; Pfeiffer and King 1983). Nutritional stress (Martin and Armelagos 1979) and osteoporotic bone loss (Huss-Ashmore 1981; Martin 1981) have been determined by microscopic analysis of remodeling in human bone from archaeological sites. Data on bone remodeling has been compared between the sexes and among different ages in association with the possible effects of the environment or genetic differences (Ericksen 1980).

Micromorphological variation revealed by microscopic analysis of thin sections of human bone from three archaeological populations was attributed to differences in diet (Richman et al. 1979). An increase in remodeling rates has been noted in the shift from hunting and gathering to agriculture (Stout 1978). It has been suggested that it may be possible with further research to detect histomorphometrical patterns that coincide with, or are typical of, specific methods of subsistence

(Stout and Simmons 1979).

Microscopic analysis can corroborate macroscopic diagnoses of pathological conditions such as osteoporosis or infectious lesions (Martin 1981) and can verify whether histological structures have actually been preserved in bone which appears normal (Stout and Teitelbaum 1976a; Stout 1978).

Microradiographs of thin sections of bone have been used to determine the distribution and relative concentration of bone mineral. Highly mineralized areas are radiopaque and appear white or off-white in microradiographs in contrast to the areas of low mineral content which do not prevent penetration by x-rays and therefore appear off-black (Jowsey et al. 1965; Stout and Simmons 1979; Huss-Ashmore et al. 1982).

Estimates of age and information about the general health of ancient populations obtained from the histological study of thin sections of bone can be compared and related to aging and sex. This information can supplement the findings obtained from the macromorphological study of bone and other disciplines to provide an overall perspective and a more complete picture of the archaeological site and its people.

The research described in this thesis will examine the micromorphology of prehistoric human skeletal samples from Portugal for such information as can be obtained about health, age at death, and possible sexual differences. Data from both macromorphology and micromorphology will be used. The general osteological analysis of the Moita skeletons was undertaken by Dr. M. Jackes of the University of Alberta who provided stature

estimates for each specimen based on the Trotter and Gleser formulae as well as the data on the Nordin Index which is an estimate of cortical thickness measured from radiographs. Jackes also determined the sex of each femur by using step-wise discriminant analysis (see Lubell and Jackes 1985).

The very features of bone which assist in their preservation make thin section preparation difficult (Stout and Teitelbaum 1976a). Even when embedded in resin, hard brittle undecalcified bone may fracture during sectioning (McQueen et al. 1972) and if bone is decalcified the mineral stages cannot be studied (Stout and Teitelbaum 1976a). An additional problem is that ancient bone may disappear if decalcified or may disintegrate during hand grinding to reduce section thickness (Blumberg and Kerley 1965).

Since undamaged good quality thin sections of bone are often difficult to obtain, even from well-preserved bone (McQueen et al. 1972; Hancox 1972; Drury and Wallington 1980), several techniques were tested on experimental segments of archaeological bone to determine the best method of preparing thin sections from prehistoric human bone. Thin sections of cortical bone were prepared from 9 mm bone cores extracted from the anterior midshaft of the Moita femora. Microscopic analysis of the sections was undertaken in the present research to obtain estimates of age at death and indications of biological health in these Mesolithic hunter-gatherers.

CHAPTER II

MATERIALS AND METHODS

The Moita Skeletal Materials

The sample used in this study consisted of cortical bone cores 9 mm in diameter extracted from the anterior midshaft of femora (see Figure 1, page 11) during the 1984 research season directed by Lubell in Portugal (Lubell et al. 1984). The sample consisted of cores from 24 left femora and 8 right femora. Data derived from the analysis of the Moita femora can be found in Table 1 (page 148). The individuals represented by the 32 intact cores included 20 males, 11 females, and one individual of unknown sex. Two males and two females in the sample have both right and left femora. Seven additional cores were not suitable for analysis or measurement because of damage. Bone core samples were removed from all left femora in order to maintain consistency since commingling of the skeletons had occurred during earlier storage (Jackes pers. comm. 1985).

A high-speed rotary drill fitted with a diamond-studded tungsten steel bit was used for removal of the cores (Jackes pers. comm. 1985). The bit was water-cooled during the entire procedure.

The femoral bone cores were taken from skeletons recovered at Moita do Sebastião [7080 $\pm$ 130 BP (H2119/1546)] one of many prehistoric shell midden sites that have been discovered in Portugal. Moita is located along the south bank of the Muge River (Figure 2, page 13) 3 kms from its junction with the Tagus in

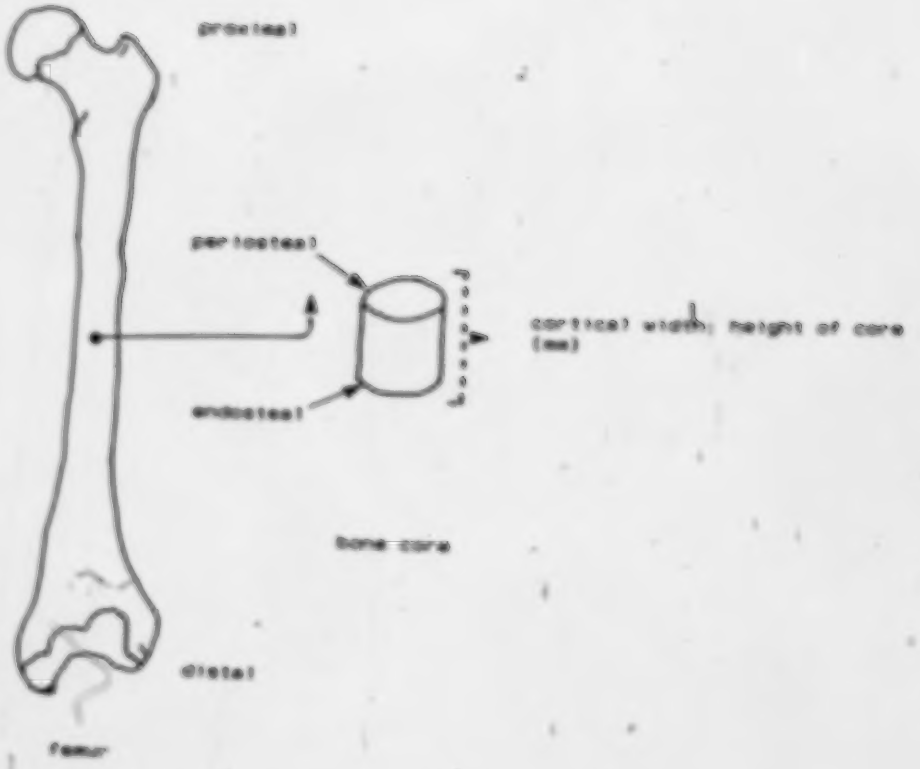

Figure 1. Diagram illustrating core extraction site at anterior midshaft of left femur.

south-western Portugal. The site, located on a bluff overlooking the flood plain, has been destroyed but at one time consisted of a mound covering 300 m^2 containing archaeological deposits 2.5 m thick. The lowest levels with archaeological deposits were 22 m above sea level (for a summary on Moita and other Muge sites see Roche 1972).

The first excavations at Moita were conducted by Ribeiro in 1880. In 1892 de Paula e Oliviera directed further excavations at the site. The skeletal material from these two excavations, presently housed at the Geological Survey in Lisbon, was analyzed in 1984 (Lubell et al. 1984). In the most recent excavations, from 1952 to 1954, Roche and Veiga Ferreira recovered additional skeletons from the lowest levels in the same site. This material is stored in Porto.

The site contains evidence of both land and estuarine animals. Shells of clams (Scrobicularia plana), cockles (Cardium edule), and mussels (Mytilus edulis), land snails (Helix) and crabs along with the bones of fish and birds represent the non-mammalian sources of subsistence. A variety of terrestrial vertebrates were included in the diet. The skeletal remains of otter, rabbit, hedgehog, pig, deer, and wild cattle have all been identified. Stable isotope analyses of human rib samples from five skeletons at Moita has suggested a diet consisting of both terrestrial and marine animals (based on delta ^{13}C and delta ^{15}N values, Lubell et al. 1986a).

Further analysis of the faunas is presently in progress, a preliminary analysis of 1184 mammalian bone fragments by A.

Figure 2. Map of Portugal showing the location of Moita, the Muge shell midden research site.

Lentacker (1985) of the Geological Institute, Rijksuniversiteit-Gent, Belgium has revealed that 88% are represented by 4 genera: rabbit (Oryctolagus cuniculus 33.4%), red deer (Cervus elaphus 22.4%), pig (Sus scrofa 20.0%), and the aurochs (Bos primigenius 12.2%).

The flora indicates pine woodland. Pinus pinea (Stone pine) was provisionally identified by analysis of charcoal found in two Muge sites (Roche 1972:86).

The lithic industry is based principally on flint with some rare quartzite. The majority of the flint artifacts consist of flakes with microlithic denticulates, geometrics and blades forming most of the worked specimens. The material excavated in the 1950s by Roche and Ferreira was dated at 7350 ± 350 BP (Sa-16) and 7080 ± 130 BP (H2119/1546) (see Roche and Ferreira 1972-1973). New radiocarbon dates for Moita do Sebastião have recently been obtained on bone collagen from skeletons excavated in the 19th century. These five new dates range from 6810 ± 70 BP (TO-135) to 7240 ± 70 BP (TO-131), confirming the original dates (Lubell et al. 1986b).

Humans were occasionally interred in the clean sand below the food debris composing the shell midden (Roche and Ferreira 1972 - 1973), however, it appears that most of the Moita skeletal remains now in the Geological Survey in Lisbon were buried within the shell midden deposits. Records contemporary with the earlier excavation are lacking.

Methods

The choice of techniques to be used in preparing thin sections from hard tissue, such as archaeological bone, is dependent on the condition of the bone itself. Several techniques were tested in order to determine the most suitable method to use with ancient skeletal material. An important aspect of a thin section is an even, flat surface without irregularities which could affect resolution of the morphological features to be assessed (Ortner and Putschar 1981; Jowsey et al.1965).

The archaeological bone used in testing the techniques came from two widely separated geographical areas. The two areas have coastal climatic conditions and both samples were removed from shell middens. One sample consisted of the distal segment of a femur from Crescent Beach (DgRr 1) in British Columbia. It was surface material collected in 1973 and has been dated at approximately 2000-3000 BP (Beattie pers. comm. 1985). The other bone sample was a four inch portion of a humerus from the Muge collection in the Anthropological Institute at the University of Porto, Portugal. This bone was dated at approximately 6000-7000 BP. Testing was performed on undecalcified, unembedded, and unstained bone. Although these two archaeological samples of human bone were used in testing the various techniques, additional osseous material was available if required. This material consisted of human ribs from Aïn Misteheyia, Algeria, a Capsian open-air shell midden site, dated at approximately 9000 BP (Meiklejohn et al. 1979).

PREPARATION OF THIN SECTIONS

A. The Archaeological Test Material

Transverse sections approximately 2.5 cm wide were removed from each length of bone with a medium-toothed hack saw. An electrically powered low speed saw, the Buehler Isomet (model No.11-1180), was used in the osteology lab in the Department of Anthropology to cut bone wafers from the bone samples. This saw has a circular blade edged with diamond-impregnated abrasive that was designed for metallurgical work on other hard material but it cuts bone equally well. The 12.7 cm by 0.031 cm size blade was used in removing all bone slices.

Buehler Isocut, an oily fluid, was used as the lubricant and cooling agent. It was added to the reservoir pan immediately below the diamond-edged wafering blade to a level which immersed the lower edge of the blade to a depth of 0.6 cm. As the blade rotated during cutting, the lubricant was carried upward on the rim to the bone sample where it effectively prevented the generation of excessive heat that might have damaged the sample. Bone dust was never a problem as the Isocut Fluid washed away the bone debris as it collected.

Each thick sample was clamped into a chuck and secured to the support arm of the Isomet saw. The micrometer was initially adjusted to position the sample for removal of the thinnest possible bone wafer from the thick sample. Setting the cut-off mechanism to deactivate the saw by a decrease in weight, slightly before completion of the cut, prevented burring of the sample.

Burring occurs when the bone wafer detaches from the main bone section before cutting is completed. This results in the removal of a tiny sliver of bone from the outer surface of the thick sample in the chuck. Unless this small sliver of bone is carefully removed from the bone wafer it will also result in the removal of bone from the surface.

The saw was activated and the support arm, in position over the wafering blade, was manually lowered until the bone sample came into contact with the rotating blade. This was the first cut in obtaining a parallel-sided wafer of bone. The micrometer was then readjusted for removal of bone wafers measuring approximately 500 um in thickness. The revolving blade was set to cut at a speed of 80 rpm for the most recent bone material, however, because of the flaking tendency of the older bone sample, the speed was reduced to 68 rpm. A weight of 75 grams was carried on the support arm while the counter balance weight was positioned to maintain a light load in order to minimize the possibility of surface damage to the bone during cutting. This weight combination resulted in even cutting with little or no damage to the bone specimen other than the occasional occurrence of flaking from the periosteum and outer unprotected edges. Heavier weights and faster cutting speeds decreased cutting time but increased the amount of bone damage. It became apparent that these problems were difficult to control or eliminate in unembedded bone.

After the parallel-sided bone wafers were removed they were agitated in a mild detergent solution to clean them of any

adhering bone debris and the oil from the Isocut Fluid. They were then thoroughly rinsed in distilled water before proceeding to the grinding and polishing phases.

Grinding

Because of problems in obtaining quality thin sections from undecalcified and unembedded bone, several grinding and polishing techniques were tested. One method of reducing the thickness of the bone slices involved the use of the Buehler Polisher/Grinder model No. 49-1650. This model was connected to an external water supply which was directed onto the grinding and polishing plates through a swing spout on the model. During grinding and polishing the flow of water used for lubrication and cooling was controlled by a knob on the machine. The speed control was set at 150 rpm (0.3 amps) during grinding on the 45 micron plate. A lower speed and light finger pressure were applied to the sections during this process in order to minimize the possibility of fracturing or damage to the bone section.

Several methods were used to hold the bone slab on the grinding plate as it rotated. One method was a technique discussed by Frost (1958). A 1.27 cm wide strip of waterproof sandpaper was wrapped around the narrow width of a 7.62 cm x 2.54 cm glass slide. With the rough side out, and holding the strip ends between the fingers, the slide was placed on the bone slab to hold it on the turning plate. In an alternative method, the finger pads were used to hold the slab on the plate during grinding, but when the slab became thinner the sandpaper

technique was of definite assistance. The finger pad method of holding the section resulted in burning skin abrasions to the fingers. No discomfort was felt until later because of the cooling effect of the water used during grinding. Another method of holding the wafer against the rotating plate involved positioning the slide with sandpaper so that it was held with one end outside the periphery of the grinding plate while the index finger of the other hand applied light pressure to the other end of the slide during rotation of the plate. Although skin abrasions were avoided the screw attachment on the side of the plate struck the fingers on each rotation. It was impossible to hold the slide and bone slice in position, though this manner of holding the slide would prove successful if the slide were longer.

A combination of two techniques was utilized in grinding the thick sections. In the first phase, the finger pads held the section on the rotating plate. When the section was reduced in thickness the sandpaper and slide method was used. As grinding proceeded the section was turned frequently to grind both sides and to retain parallel sides. When the section was reduced to approximately 120 um, grinding was halted and the 15 micron polishing plate was used to give a light polish to both sides of the thick section. Because both sides of the thick section were given similar treatment there was no difficulty in deciding which side of the section should be mounted with the polished side down onto a glass slide. With one side mounted and polished, subsequent handling was decreased since only one surface needed

to be polished further until the thin section stage was reached. This was an important consideration, particularly with this ancient archaeological bone as it had already given some indication of its extreme fragility.

At this point the thick sections were examined under a dissecting microscope. Light yellow discolored areas were observed in the bone. In an effort to remove this color the thick sections were immersed in an oxidizing solution. Ordinary Javel household bleach containing 5.25% sodium hypochlorite (NaClo) was used for this purpose. It was diluted with distilled water to obtain a 20% solution by volume. After remaining in this solution for 30 minutes the sections were removed and agitated in distilled water to remove all traces of the oxidizing solution.

Mounting

It was difficult to obtain quality thin sections from unembedded material. The bone tissue sometimes disintegrated or fractured in the final steps of thin section preparation using the Buehler polishing plate. Mounting the section onto a glass slide with a supporting medium that protected the section during the latter stages of thickness reduction was important in decreasing the effects of this problem.

Diatex is a synthetic mounting resin that was used for this purpose, however, it is not miscible with water. Before the section could be mounted onto a glass slide with Diatex, water had to be removed from the bone tissue by immersion in a series of alcohols of increasing concentrations (Bancroft and Stevens

1977). Dehydration was obtained in 70%, 90%, and 100% concentrations of Ethanol. The ground thick sections were immersed in each solution for a period of 20 minutes. After the 100% Ethanol the sections were immersed in xylene for approximately 15 minutes. Xylene acts as a clearing agent and is miscible with both 100% Ethanol and Diatex (Bancroft and Stevens 1977). Xylene removes the alcohol from the bone and increases the translucency of the bone tissue. Alcohol must be removed from the tissue as it is not miscible with mounting media such as Diatex or Permount.

After the xylene bath the sections were ready for mounting on clean glass slides. Three drops of Diatex were placed on the slide and the section was carefully lowered onto the drops. Pressure was applied on the section with forceps for approximately 2-3 minutes until the Diatex began to thicken. Because Diatex dries slowly the mounted sections were allowed to dry for 48 hours before final polishing.

Polishing

A speed of 50 rpm (0.1 amp) was used for polishing on the 15 micron plate. The slow speed was required because it was almost impossible to control and hold the section and slide on the plate while it rotated. At faster speeds the slide and its section were often loosened from the finger grip and rotated to the periphery of the plate. An attempt was made to hold the slide with sandpaper but, unfortunately, this was not possible at this stage. The sandpaper was thicker than the desired thin section;

consequently, during polishing the sandpaper was destroyed first and the problem remained.

More control was obtained in the following manner. The water flow was increased until the glass slide floated but contact with the rotating polishing plate could be maintained. Light pressure with the finger pads on the glass slide maintained control and in addition prevented skin abrasions during production of the thin section. Polishing continued until the thin section was approximately 100 um thick and the surface appeared free of scratches. Thickness was estimated by first focusing the microscope on the upper edge of the bone in the thin section, next on the lower edge, and then measuring the distance (um) traveled between the two edges as defined on the micrometer scale of the microscope. The slide with its polished thin section was immersed in xylene to dissolve the Diatex in preparation for remounting. When the thin section was placed in xylene it increased in transparency. Because of section fragility extreme care was exercised when transferring the section to a clean glass slide for mounting with Diatex. Small paint brushes with long soft bristles were helpful for this purpose as in this way one could avoid handling or damaging the sections with the fingers or forceps. The small sections cling to the wet bristles thereby facilitating their transfer through other media, such as xylene, and onto glass slides. As the sections came into contact with other solutions they appeared to be attracted to the new media and slipped or floated off the bristles very easily. They did not require dislodging.

Remounting

The thin sections were positioned in the centre of a fresh glass slide with the outer periosteal edge parallel to the long edge of the glass slide. This alignment is important for microscopic viewing, otherwise continuous readjustment of the microscope stage becomes necessary each time a new field is assessed. Two small drops of Diatex were placed on the thin section with a glass rod and then the cover slip was lowered onto the section at a 30 degree angle to prevent trapping air bubbles under the cover slip (Galigher and Kozloff 1971). Air bubbles can distort the image of the micromorphological features in a thin section, causing difficulties in their interpretation.

No pressure was applied on the cover slip so that the Diatex spread evenly over the entire area. Application of pressure tends to draw air under the cover slip when the pressure is released. Much more serious is the fact that the thin section floats out of position, occasionally moving right to the periphery of the cover glass. When this occurs the section must be reimmersed in xylene to dissolve the Diatex and then it is remounted.

The mounted sections were set aside to dry before later microscopic examination. They must be maintained in a flat position until dry because if they are stored on edge in a slide box while still wet the section will slip off the slide and may be lost. Very few of the thin sections in this testing phase succeeded in reaching the remount stage in good condition. Section fracturing and disintegration occurred with loss of even thickness because some areas of the bone appeared to grind down

faster than other areas. Flaking of unprotected edges was a constant problem, therefore, the sections of bone decreased in area from the outer edges.

One major problem was the non-adherence of many sections to the glass slide in spite of using fresh clean slides and allowing them to dry for longer than 48 hours. Diatex was incapable of holding these bone sections on the slides. Varying the pressures applied to the slide during polishing or the use of different speeds did not assist in holding the section on the slide until the thin section stage was reached. The use of frosted slides with Diatex was successful in holding the sections; however, the sections still had a tendency to fracture and crumble before the desired thinness was achieved.

Another method of preparing thin sections was attempted with similar results. The thick section was first ground and then polished to a thin section without benefit of prior mounting. Care in holding the sections on the plates and using a slow rotation speed maintained fairly even thickness and avoided major skin abrasions of the fingers. Sandpaper was also placed on the section with the flat of one finger but it was very difficult to judge thickness reduction in spite of frequent observation and turning. Grinding and polishing on the stationary plates resulted in the same problems as encountered before.

Because the quality of the thin sections was not always acceptable another experimental method that combined both grinding and polishing at the same time was utilized. This technique was modified from Bancroft and Stevens (1977) and

involved the use of frosted plate glass. The thick slab was initially hand ground on a frosted plate with water and the finger pads were used to manipulate the section in a circle over the plate. When the section was reduced in thickness the top plate was placed over the section and moved in a circular motion to accomplish both grinding and polishing until the desired thinness was obtained.

One advantage of this method is that the section remains in full view at all times and progress in tissue reduction can be observed. A possible disadvantage with this method is the collection of grinding debris which may not be completely removed by flooding the area with fresh water. Frequent turning of the bone slab was not required after the initial stages because the section alternated in clinging to either the top or bottom plate. Polishing of both surfaces occurred during reduction in thickness. Suction build-up between the plates was sometimes a problem but in most cases the addition of more water alleviated the situation.

Further preparation of the thin sections before mounting followed the same schedule as previously discussed. Each section was immersed in graded strengths of Ethanol (70, 90, 100%) for 20 minutes each, followed by immersion in xylene for approximately 10 minutes before final mounting on slides with Diatex and cover-slipping. If the thin sections are not as transparent as expected after the xylene bath, dehydration may not be complete and they should be returned to 100% Ethanol (Drury and Wallington 1980). Clearing in xylene can then be repeated.

Since these grinding and polishing techniques produce rather unpredictable results with ancient archaeological human bone, great care must be exercised if these methods must be utilized.

B. The Moita Research Bone Cores

The subject of resin embedding was discussed in reference to the cores and the problems of flaking and disintegration during thin section preparation of the test material. Several types of analysis planned for the remaining core material, after a thin section was removed, required unembedded material. Embedding of the cores was therefore eliminated and thin section preparation was attempted with the grinding and polishing plates. Thin sections were prepared from undecalcified, unembedded and unstained bone cores.

When the chucks that were available for holding the bone cores during sectioning were examined it was discovered that they were not suitable for the purpose. It would have been impossible to immobilize and securely position the cores in the v-shaped groove of the chuck. Any movement of the core during sectioning might destroy the core or require a new cut, resulting in wastage of the bone core material. Therefore a chuck was modified so it would securely hold each core during cutting.

A 9 mm semi-circle was precision-ground on the facing edge of both halves of the chuck so that each semi-circle was an exact mirror-image of the other when they were fitted together (Figure 3, page 27). Modification of the chuck in this manner permitted

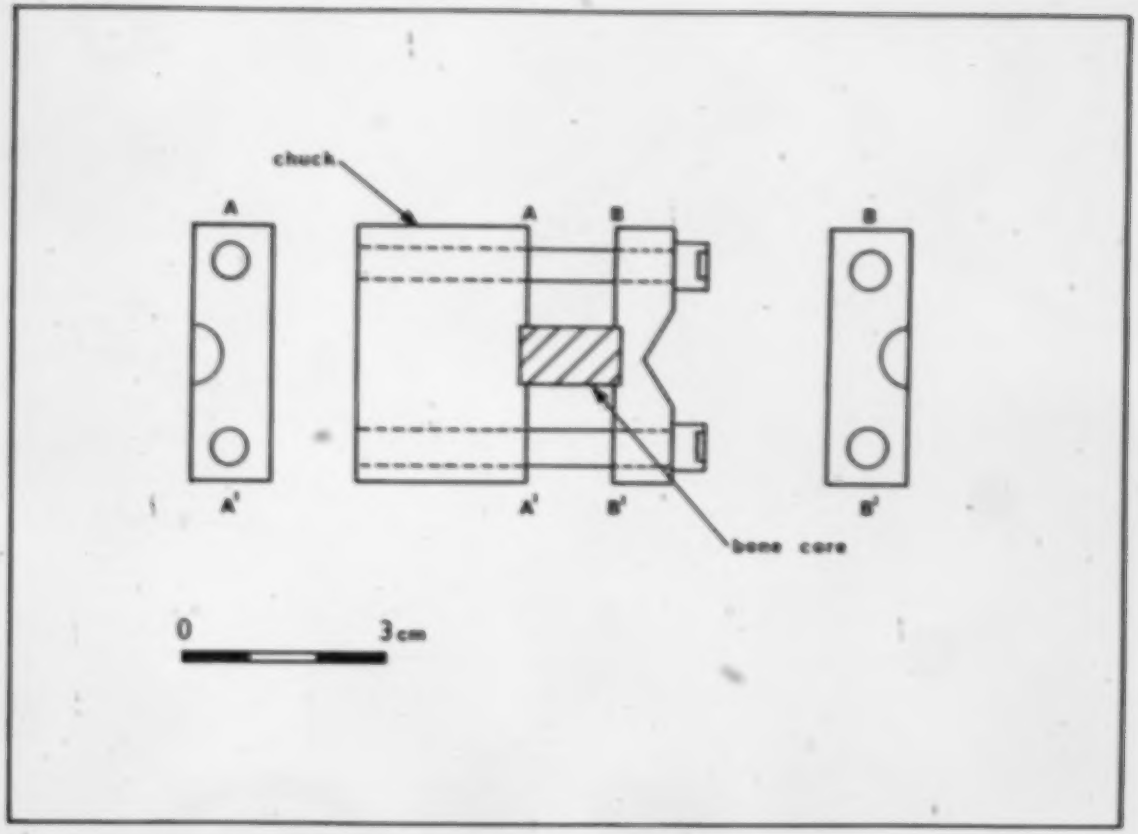

Figure 3. Diagram of chuck (hold down type). Modified to prevent potential damage to bone core by holding sectioned portion securely in the 9 mm semi-circle after cutting is completed.

the inner half of the core to be held firmly while a bone slab was removed from the exposed outer half of the core. In order to obtain parallel-sided wafers each core was clamped into the modified chuck, taking care that pressure from tightening the clamp was adequate to hold the core but not sufficient to cause fracturing.

A slight error in positioning the core during sectioning of the first test core prevented obtaining a cut that was transverse to the longitudinal axis of the femur. Cross sections are necessary to expose fully the bone microstructure since the osteons are arranged parallel to the longitudinal orientation of the femur (Jaworski 1983). To increase accuracy in obtaining a right-angled cut across the striations (grain) on the periosteal surface and transverse to the longitudinal axis of the femur the following procedure was implemented.

The cores were positioned in the chuck semi-circles, with minimal tightening of the clamp screws, and viewed under a dissecting microscope. To minimize the possibility of losing the periosteal surface through flaking, each core was placed in the chuck so that the periosteal surface would be cut first. If burring should occur near the end of the cut it would affect the endosteal surface and not the periosteal areas to be used for determining age.

The straight edge of a right-angled triangle was placed along the long straight edge of the chuck and the right- angle of the triangle was aligned with the striations (grain) on the periosteum. The striations or grain runs parallel to the

longitudinal axis of the femur (Frost 1969). Occasionally, when these striations were difficult to observe, a slight surface brushing with distilled water was enough to bring out the necessary visual detail. When alignment had been obtained the screws of the clamp were firmly tightened and then the alignment was rechecked for accuracy. Many times the procedure had to be repeated as the final tightening of the clamp sometimes disturbed the alignment.

Sawing

Once a core was aligned and securely positioned, the chuck was attached to the cut-off arm of the low speed saw (Buehler Isomet) that was used in the original testing technique. The micrometer was adjusted to position the core over the blade for the removal of an outer slice, then the cut-off mechanism was set to deactivate the saw near the finishing steps of cutting to prevent burring. The speed was set at 135 rpm and the loaded arm was gently lowered onto the revolving blade. Readjustment of the micrometer was necessary at the beginning of each cut in order to obtain the desired thickness of the wafer. Only one parallel-sided wafer measuring 500 um was removed from each core. The remainder of the core and bone material removed in the initial slice were reserved for future analysis. Despite using an identical speed in removing all wafers, the cutting time varied between 1-8 minutes and was not dependent on the thickness (height) of the core.

After the bone wafers were removed from the cores they were

subjected to the following procedures. Both the remaining core and the wafer were agitated in a mild detergent solution to remove the oily film of the Isocut fluid and then they were rinsed in distilled water. Each core was set aside to dry before storage in a small plastic bag lined with absorbent paper.

Grinding

The bone wafer was ground on a 45 micron grinding plate with liberal amounts of flowing water. Both sides were ground and lightly polished until the section was approximately 100 um thick and then the section was re-rinsed in distilled water. Because of the discoloration or ash-gray colored areas in the original test material and because these wafers appeared to be discolored in a similar manner they were placed in a 3% solution of hydrogen peroxide for bleaching. The Javel bleach solution was not used as it had not lightened the stain in the original test bone as effectively as desired. They remained in the hydrogen peroxide solution for 24 hours. This step was followed by a rinse in distilled water before ultrasonic cleaning in a mild detergent solution for 30 minutes. During ultrasonic cleaning the thin sections a slight amount of white precipitate formed on the bottom of the beaker. In several cases one or two tiny black granules were observed. After ultrasonic cleaning sections were given a final rinse in distilled water to remove all traces of detergent.

Dehydration

Dehydration was accomplished in a graded series of increasing strength alcohol. Each slab was placed in solutions of 70, 90, and 100% Ethanol (#1) for 20 minutes. To ensure complete dehydration the thick sections were put into a fresh solution of 100% Ethanol (#2) for a further 20 minutes. After five sections had been dehydrated, the solution of Ethanol #2 was rotated downward to replace #1. The original #1 solution was discarded. A fresh solution of 100% Ethanol (#2) was prepared for use.

Dehydration was followed by clearing in xylene for 20 minutes. The thick sections were removed from the xylene with a small brush and mounted on clean glass slides with Diatex. Light pressure was applied to the sections with forceps until the Diatex began to thicken. This usually occurred in approximately 2-3 minutes. The mounted slides were set aside to dry on a flat surface for at least 48 hours.

Polishing

The Buehler 15 micron polishing plate was used to polish the thick sections. The slide with the thick section was held on the revolving plate with the finger pads as previously discussed. A speed of 50 rpm (0.1 amp) was used throughout all polishing. The non-adherence of Diatex and thin section to the slide continued to be a problem. In these cases, hand polishing on one frosted plate glass was necessary to complete thin section preparation.

The thin sections were soaked in xylene until the Diatex dissolved. They were then remounted in another resin, Permount,

on fresh glass slides. Although the sections appeared transparent at this stage, later inspection revealed dense areas within the sections.

Differential hardness encountered during grinding and polishing caused difficulty in obtaining thin sections. The harder areas within the bone sections did not reduce in thickness before the softer areas began to disintegrate. If polishing was discontinued before this point was reached, the denser white mottled regions in the section prevented observance of the microstructures.

Embedding

Due to the unsatisfactory quality of most of the thin sections and the inability to discern the microscopic anatomy of the osteons and other structures it was decided to cut fresh sections and embed them in a plastic-embedding medium.

A bone wafer, measuring one millimeter in thickness, was removed from each core. The wafer was then washed in a mild detergent to remove the oily film, rinsed in distilled water, immersed in 3% hydrogen peroxide for 24 hours, ultrasonically cleaned and finally rinsed in distilled water. Dehydration in alcohols in a graded series of increasing concentration and clearing in xylene completed the preparation for embedding in Ward's Bioplastic, a liquid synthetic resin that becomes crystal clear as polymerization or curing progresses. It was hoped that embedding would prevent disintegration of the bone and improve resolution of the micro-features. There was concern that the ash-

gray discoloration observed in the sections might have been caused by the oily amber-colored Isocut Fluid. In order to investigate this possibility dry rib segments from Aïn Misteheyia and bone wafers removed from the Crescent Beach femur, using distilled water as the coolant, were also embedded in Ward's Bio-Plastic.

Sawing

After the embedding material had cured for 48 hours the blocks containing the bone slabs were sufficiently hard to allow sawing. The Buehler Isomet Low Speed Saw was fitted with a 7.62 cm diamond-edged wafering blade to remove thin sections from the embedded blocks. Distilled water was used as the cooling agent.

Thin sections of approximately 80-100 um were cut from the blocks. In some cases thicker sections were cut because of fracturing. These were then hand ground between frosted -glass plates. Once again there was a problem with bone disintegration. It was impossible to obtain sections thinner than 80 um. The thin sections were cleared in xylene and then they were mounted on glass slides with Permount in preparation for microscopic assessment.

Equipment

A Wild Leitz SM-Lux microscope was used in this research. It has settings that allow section visualization under ordinary light or it can be set for use as a fluorescence microscope. The fluorescence microscope was used to view the thin sections under

illumination with blue light, between approximately 380 and 420 nm. This wave length elicited a green fluorescent hue in the bone tissue. All the sections were examined with a 10x ocular lens and a 10x objective (100x) with both types of lighting as a preliminary step in investigation. Various aspects of the thin sections were photomicrographed with both types of lighting to provide a permanent photographic record for examination during later phases of the research. A camera with the Wild Leitz MPS 55 Photoautomat system was used in all photomicrographic procedures. The photographic film used with tungsten microscopic lighting was Panatomic X, 32 ASA, 35mm black and white film. The transparency slides were obtained with Ektachrome 100 daylight 35 mm color film and tungsten lighting. Ektachrome 400 daylight color film was used for transparency slides with fluorescence lighting. Several measurements were taken on the dry bone cores (Table 1, Appendix). The cortical thickness (height) of each bone core was measured to the nearest 0.001 mm with a Vernier Scale on an outside micrometer. Measurements were taken from the periosteal rim to the cortical-endosteal border through the center of each core. The core weight and diameter were not obtained until after the first section had been removed. The remaining portion of the core was weighed to the nearest 0.1 gram on a Brainweigh™ B 5000 located in the osteology lab in the Department of Anthropology at the University of Alberta. Core diameter, measured to the nearest 0.001 mm, and core thickness (h) were used to estimate core volume. Core volume was estimated with the measurement of core diameter and core thickness (h) using the

formula for determining the volume of a cylinder, $\pi r^2 h$, where h represents the thickness or length of the core and r is the radius. The density (gm/cm^3) or mass per unit volume of the bone cores was calculated by dividing the core weight by the core volume. This method of determining volume and density of bone cores was developed by Thompson (1979,1980). His entire core technique was not followed as a grid for point counting used in determining the area of microstructures, such as osteons, was not available.

Microscopic Measurements

All assessments of the microstructures in the thin sections of bone were made using a 10x ocular lens and a 10x objective (x100). A square-ruled grid fitted into the microscopic ocular lens assembly was used for the quantification of microstructural elements. Total grid area measured 0.92 mm^2 at the level of the section. The grid contained 144 small squares (12x12) each measuring 0.08 mm. Each side of the grid measured 0.96 mm.

The Ahlqvist and Damsten (1969) method for estimating age by counting osteons and osteon fragments was utilized. Osteons and osteon fragments increase in number as an individual increases in age. The outer periosteal one-third of the cortex is said to be associated with such age changes and has fewer changes resulting from bone resorption (Kerley 1965). The square-ruled grid was positioned along the periosteal border and four microscopic fields, spaced at equal distances across the thin section, were assessed for the number of osteons and osteon fragments. Each

square in the grid that was more than half filled with osteons and osteon fragments was counted. Normal microscopic lighting was used in this portion of the research. After several attempts to obtain age estimates by counting osteons and osteon fragments this method was discontinued. The problem is discussed later in more detail under age determination (page 59), but see Plates 2 and 3 page 53.

The fluorescence microscope, using blue-violet excitation, was used to count all resorption spaces in the bone. No other lighting was used so only the resorption spaces could be observed. There was no distraction by other microstructural features. The resorption spaces appeared dark in comparison to the adjacent bone because they contained no fluorescent material. In fluorescence microscopy, a sample is illuminated with light of one wavelength. This primary exciting light is first absorbed by molecules in the sample which then re-emit light of a longer wavelength to produce an image. If the light does not strike anything, it does not cause fluorescence (Bloom and Fawcett 1975; Nairn 1976).

All bone resorption cavities were counted using the same square-ruled grid as previously described. The total number of cortical resorption spaces in the outer periosteal one-third, the middle one-third, and the inner cortical-endosteal one-third were counted twice (Counts one and two) across the entire width of each section (Table 2, page 149 and Table 3, page 150). The total number of resorption spaces counted in each one-third were added together and then divided by the number of fields assessed

to give the average number of bone resorption spaces in an area covering 0.92 mm^2. Percentages were calculated for each one-third of the cortex to determine their contribution to the observed bone porosity.

A separate count on the inner endosteal one-third (Count three) was also made. In this count resorption spaces smaller than approximately 80 um or larger than 400 um were not counted. Four microscopic fields with resorption spaces measuring between 80 um and 400 um were counted. The four fields were spaced apart as equally as possible but in some instances the field had to be moved when a resorption space nearly filled the entire grid area. The average number of resorption spaces per 0.92 mm^2 in each section was obtained by adding the total number counted in the four fields and dividing that number by four.

Scanning Electron Microscopy

A thick section of the humerus from Porto and five rib segments from Lisbon were analyzed for trace elements that might assist in identifying the bone stain. The equipment used for the analysis was the Cambridge Stereoscan 250 with a Kevex 7000 energy dispersive x-ray in a scanning electron microscope. This equipment is located in the Geology Department at the University of Alberta.

Chemical Staining

Several thin sections were selected for treatment with chemical stains. A thin section of the Porto humerus was stained

with Perls' Prussian Blue to detect the possible presence of iron (Drury and Wallington 1980). Ehrlich's haematoxylin and eosin were used to outline the cement lines (Cook 1974; Wheater 1979; Drury and Wallington 1980; Bancroft and Stevens 1977) although the glycoprotein content in the cement lines of fresh bone may not be preserved in archaeological bone. Thin sections from the Porto humerus and a Lisbon femur were stained with Harris' haematoxylin and eosin to show areas of calcification (Drury and Wallington 1980; Bancroft and Stevens 1977). A thin section from the Lisbon material was stained with the von Kossa stain to demonstrate calcified areas or deposits (Drury and Wallington 1980). It was counterstained with van Gieson's for collagen and lamellar bone (Drury and Wallington 1980). One thin section was decalcified with Formic acid-Sodium Citrate solution in an attempt to remove the ash-gray discoloration and reveal the microstructural features. Since the lamellar pattern of collagen is retained in decalcified archaeological bone (Stout and Teitelbaum 1976a; Stout 1978) it may be possible to determine the number of osteons and osteon fragments in a thin section and in this way obtain an estimate of the age at death.

CHAPTER III

RESULTS

Core Appearance

The cores were examined under a dissecting microscope to investigate their general overall appearance. Most of the cores had thin lines or striations encircling their entire circumference. These impressed lines may have caused the fracturing and flaking of chips from several cores during later sectioning. As a result of this problem new sections had to be cut on several cores. Some cores had light or dark amber colored areas while others had varying degrees of blackening. Two thick slices from the cores with extensively blackened regions fractured after sectioning.

The occurrence of these linear marks may have resulted from several factors. Whether or not these were compression lines resulting from the weight of soil overburden during their long interment period is difficult to judge. The method used in obtaining the cores is a factor that has to be considered especially since their extraction was extremely difficult. The femora were semi-fossilized and within a hard concrete-like soil matrix (Jackes pers. comm. 1985). Although the high speed drill was water-cooled during core removal it is possible that the leading edges of the drill became excessively warm resulting in the amber and black colored areas.

The periosteal and endosteal surfaces of the cores had white salt-like crystal deposits varying in appearance from fine to

coarse grains. Areas of gray crust were also present. Several of these white-or-gray crusted areas had a baked-mud appearance with cracks outlining roughly hexagonal areas. Black many-sided crystals were more common on some cores than on others as were the black pigmented areas. The clear crystals were triangular or elongated angular structures. Some crystal forms appeared like long sharp needles. Such a thin sharp pointed crystal had sufficient firmness to penetrate the skin. There were areas of heavier black coal-like seams broken into small rectangles on the surface of some cores. Clusters of small black crystals were also noted on several cores.

Thin Section Quality

Thin sections from the femur from Crescent Beach (2000-3000 BP) were reduced in thickness less rapidly than those of the Porto humerus (c 7000 BP). This may indicate differences in the mineral or organic content of the two bones. The Porto bone segment was particularly important in the testing. It was important to obtain information on hardness, flaking, sawing and section quality before work commenced with the research bone cores.

The results of these trial and error tests indicated that thin sections would not be easily obtained from bone material of such great age. Section quality was similar irrespective of whether the sections were mounted or unmounted during grinding and polishing. Thin sections of bone prepared by grinding between frosted plate glass produced similar results even when suction

between the two plates was controlled and light pressure was used in an effort to minimize section fracturing and flaking.

The fragile nature of the Moita bone, its variation between hard and soft tissue areas, and its propensity to disintegrate before the desired thin section was obtained, eliminated the hope of producing quality sections by grinding and polishing. It was concluded that embedding is mandatory when dealing with such fragile bone.

Better results were obtained when 1 mm bone slabs from the cores were embedded. The embedding medium protected the thin sections during their removal from the bone slabs. Other than several sections which fractured during sawing most were removed intact. It was simple to cut replacements for those that did fracture.

Visual observation of the Moita bone slabs revealed differences in surface color, and this was confirmed when the slabs were viewed under a dissecting microscope as well as when thin sections were examined microscopically. The slabs showed light yellow colored areas which appeared as dark ash-gray in color when examined on the thin sections.

The ash-gray discoloration persisted in the thin sections of the test material despite prior treatment with an oxidizing agent and ultrasonic cleaning. Similar treatment of the Moita research material produced identical results. This reduced the number of thin sections that could be used for determining age at death since the discoloration obscures the microstuctures.

Similar problems with discoloration were encountered by

Pfeiffer (1985). The removal of organic and inorganic soil contaminants that had penetrated the periosteal areas was outlined by Ubelaker (1974) in his research. His method involved treatment of the bone slabs with an oxidizing agent and ultrasonic cleaning. This cleared the tissues sufficiently for microscopic examination of the osteons, and hence for determining age at death. Other workers have used sodium hydroxide (NaOH) on archaeological bone to dissolve humic residues from decomposing plants (Boutton et al. 1984). Bromoform, an oxidizing agent, has been used to improve the transparency of 500 um thick sections before study with a polarizing microscope (Ascenzi 1983).

The combination of molluscan shell, soil minerals and organic material associated with shell middens may have fostered some type of chemical reaction in the presence of water seepage which discolored the bone. Bone from all three shell middens exhibited identical ash-gray discoloration in the thin sections, and this remained even when distilled water was used as the coolant during sawing, discounting any possibility that the Buehler cutting oil was responsible for the discoloration. The British Columbian and Portuguese sites are both found in more humid climates today than the Algerian one, yet all showed similar discoloration.

From the total of thirty-two sections embedded in plastic, only one was sufficiently clear for an osteon count. The reason for this outcome is difficult to determine. This section was better preserved than the others, suggesting that burial may have been more recent than the other skeletal material or that this skeleton may have been less affected by pedogenic factors due to

its location in the burial site. If the discoloration was caused by a chemical reaction between soil-shell material and water seepage it could indicate that this individual was interred nearer the surface of the midden or near the outer boundaries. No data on the location of the skeletal remains in the site have yet been found.

Histochemical Analysis

The pale yellowish-gold discoloration (darker in some areas) on the thick sections and the microscopic observance of an ash-gray discoloration obscuring the microanatomy in the thin sections were considered as possible contributors to the different areas of hardness in the bone sections. Even though the yellow-gold discoloration had been treated with an oxidizing agent to remove the discoloration as much as possible, it was obvious that it remained. Despite the difference in appearance between the thick and thin sections it was quite possible that it was a single stain since the discoloration occurred in the same areas. The source and identity of the stain(s) remains unknown.

Iron was considered as a possible contaminant mainly because some areas of the bone were harder than others. Other research has also implicated iron as the source of a gold colored stain that obscured the microstructures and produced different areas of hardness in bone sections (Pfeiffer 1985). The idea that the ash-gray discoloration might be a blood residue which had infiltrated the bony tissue after death was also considered. Histochemical techniques were used to test for iron in the residue of

haemoglobin breakdown to haemosiderin, an iron containing pigment, or from an inorganic source in the surrounding soil.

A thin section from the Porto humerus was used in this test. Perls' Prussian blue is the classical method (Drury and Wallington 1980) for haemosiderin and inorganic iron. A positive reaction for iron was shown by the appearance of a blue colored rim approximately 0.08 mm wide on the periosteal border. The only visible substance in the remainder of the section appeared to form a loose network of connected strands without identifiable structural features. An increase in flexibility was noted while transferring the section from a xylene solution to a glass slide for mounting. This suggests that the 2% hydrochloric acid used in the Perls' Prussian Blue method decalcified the thin section. It has been suggested (Villanueva 1976) that staining methods involving the use of acid must be avoided as complete or partial decalcification of thin sections tends to occur.

Thin sections from the Porto humerus and the Moita femur (LOOD7) were stained with haematoxylin and eosin to show the cement lines between osteons. It was hoped that this would demarcate the outer limits of osteons thereby allowing an estimation of their size and number regardless of the ash-gray discoloration. Harris' haematoxylin and eosin stained the Porto section a bright pink with no blue coloration to show cement lines. The presence of occasional calcified areas were indicated by blue deposits in the section. Collagen in fresh bone stains bright pink with eosin (Drury and Wallington 1980) but it is difficult to believe that the entire Porto section would

demonstrate only a few calcified areas since other tests have indicated high calcium levels. Ehrlich's haematoxylin and eosin stained the Moita bone a light purplish-pink color with sparse purplish-blue coloring in some areas. No cement lines were observed with this stain. Although there was a slight indication of calcium deposits the results were inconclusive with these particular stains. It has been suggested that bone tissue changes in archaeological material result in poor haematoxylin-tissue reactions (Stout and Simmons 1979).

The von Kossa staining reaction is of value in demonstrating calcified tissue (Drury and Wallington 1980). The silver in the stain is thought to replace calcium in calcium carbonate, phosphate and oxalate and is shown as black deposits. With this method, heavy black deposits throughout the Moita section were interspersed with red deposits. The black deposits were more pronounced in the outer periosteal one-third than in the middle or endosteal one-third. Counterstaining with van Gieson's showed the deep red of collagen and osteoid. Although some researchers have failed to demonstrate osteoid in ancient bone (Stout 1978; Ortner and Putschar 1981) other research has revealed the presence of "collagen characteristic of osteoid" in archaeological bone (Race et al. 1972:273). The deep red in the Moita section appeared to be associated with Haversian canals or perhaps resorption spaces, but it was difficult to determine. The Moita bone section showed some areas with a yellowish-pink color which is typical for mature lamellar bone stained by the van Gieson method (Drury and Wallington 1980).

The high calcium levels reflected with von Kossa's method and with the scanning electron microscope were verified by observation after decalcification of another thin section from the same Moita femur. Use of Formic acid-sodium citrate solution resulted in the complete disintegration and disappearance of the structures in the tissue section.

The presence of calcium carbonate was suggested by the slight bubbling which occurred when the section was treated with formic acid-sodium citrate, and was probably due to the release of carbon dioxide (Bancroft and Stevens 1977). A small amount of light tan colored paste was all that remained, and when agitated even this went into solution. If collagen was present, as indicated by van Gieson's stain, it is assumed that the acid was of sufficient strength to cause dissociation of the collagen. When bone mineral is removed from fresh bone by a weak acid the bone retains its organic structure and flexibility, and it has fairly normal morphological features (Bloom and Fawcett 1975). It has been proposed (Race et al. 1972) that protein in collagen is lost in the fossilization process, and that the micromorphology of collagen is retained by replacement of these proteins with other minerals. They support this view with the fact that decalcification of ancient bone with acids may result in dissolution of the entire bone. When one undecalcified thin section of the Moita bone was viewed under polarizing light the bone clearly showed the characteristic collagen birefringence of the lamellar structure in the osteons.

Collagen shows birefringence because the collagen fibers

change direction in each successive lamella. This orientation of the fibers shows a characteristic pattern of light and dark layers when viewed with a polarizing microscope (Hancox 1972). Transversely cut fibers appear black in contrast to the lighter appearing longitudinally cut fibers.

The concrete-like matrix adhering to the endosteal borders and filling the medullary canal of the Porto humerus was successfully dissolved by 2% hydrochloric acid. After evaporation of the fluid, a precipitate containing varied crystal-like shapes formed over the bottom of the beaker. These crystals varied in color from red, gold, and copper to colorless.

SEM Analysis

Trace element analysis of these matrix crystals was undertaken with the Cambridge Stereoscan 250 equipped with a Kevex 7000 energy dispersive x-ray in a scanning electron microscope (SEM) located in the Geology Department at the University of Alberta. Elemental calcium was most abundant, and some phosphorus and trace amounts of copper and iron were detected. Analysis of a small bone sample from the Porto humerus gave similar readings for calcium and phosphorus. No conclusions could be drawn regarding the identity of the ash-gray stain nor could the reason for the different areas of hardness in the thin sections of bone be determined. Other elements, if present, were either below the sensitivity level of the equipment or the sample of bone and matrix crystals was too small.

The matrix adhering to the skeletal remains analyzed in 1984

varied from a loose sandy material to intensely hard disintegrated mollusc shells (Jackes pers. comm. 1985). The variability of the matrices was represented among five rib samples analyzed for both stable isotopes and radiocarbon (Lubell et al. 1986b; Meiklejohn et al. 1986). Radiocarbon dates confirm that the samples are contemporaneous. SEM analysis (U of A) of these ribs indicated high calcium levels and some phosphorus. The matrices adhering to the ribs are similar, indicating a clay background with aluminum, silicon, potassium, and iron, and also including calcium phosphate presumably originating from crushed shell. The variations among the matrices of these ribs cannot, on present evidence, be attributed to date and/or population. It is more likely that they are the result of differences in the chemical and physical characteristics of the midden deposits. Such physical variations in the midden deposits are clearly illustrated by Roche's work (1972:80) at the neighboring Mesolithic midden of Amoreira.

The SEM visual scan on the periosteal surface and within the pores of the Porto bone fragment displayed small oval bodies approximately 1 um long and 0.05 um in thickness (Plate 1, page 49) which have been tentatively identified as bacteria (Whitehouse pers. comm. 1986). Whether they were calcified was not determined in the original scan as they are so thin that the SEM might have given a reading for the calcium beneath them. Since the bacteria could not be analyzed while on the bone, because SEM penetrates deeper into the bone layers, an attempt was made to isolate and identify their type and determine whether

Plate 1. Scanning electron micrograph of unidentified bacteria associated with an archaeological human bone sample from a humerus in the Porto skeletal collection. Bar represents 1 um.

they were calcified or of recent origin. A new sample was obtained by scraping the periosteal surface of a bone fragment from the Porto humerus.

Although these small oval bodies were tentatively identified as bacteria in the photos of the original SEM scan there were no bacterial specimens noted in the bone scrapings (Whitehouse pers. comm. 1986). They could not be isolated, therefore, their type is unknown. The bacteria that were present may have been an isolated incident. Microscopic examination of the bottled Buehler cutting oil and a sample of the waste cutting oil with its bone residue did not reveal evidence of bacterial contamination although it was not possible to examine all of the waste residue. Even if the type of bacteria had been identified it would have been impossible to pinpoint their time of arrival at a specific stage in the history of the bone (Whitehouse pers. comm. 1986). It is possible that these bacteria were associated with decomposition following burial and that they themselves were thereafter fossilized.

A rare opportunity to identify possible disease-causing agents was lost when these bacteria could not be isolated and their type positively identified. The association of bacteria with the Porto research bone might have provided evidence of possible health problems that could have affected prehistoric populations. The identification of these bacteria as specific pathogens might have provided much needed information in this area and made an important contribution to palaeopathology. Many fatal diseases leave no identifying marks in the skeletal

framework (Sandison 1968) while the same morphological changes may be caused by different pathogenic organisms because bony tissue is limited in the number of ways it can react to pathological processes (Stothers and Metress 1975; Steinbock 1976). The bone in the thin sections showed no identifiable tissue alterations that could be attributed to disease processes although the ash-gray stain might have obscured any such changes.

Diseases of wild animals (zoonoses) were more likely to have created health problems for hunter-gatherers than epidemic or contagious human diseases because of small group size and frequent mobility (Smith 1976; McElroy and Townsend 1979). Such animal diseases can be transmitted to people and maintained in the population at continuously low levels for indefinite periods of time. Sporadic episodes of Bubonic Plague have been transmitted to hunter-gatherers in contact with wild rodents (Reed 1970). Seasonal aggregation at a site by several groups, or longer term habitation of one site by one group, presents the possibility that more serious health problems could have existed. If the Moita people were in the process of becoming semi-sedentary they may have experienced some of these health problems.

A potential risk for the Moita population at this site may have been the danger of food poisoning from contaminated shellfish. Illness in modern populations living near coastal waters has been attributed to the consumption of crabs, and marine and estuarine fish infected with the bacteria Vibrio parahaemolyticus, (Krantz et al. 1969; Mausner and Bahn (1974)

and to food poisoning from consumption of other marine animals such as mussels, crabs, and clams, which have acquired poisonous toxins through eating toxic marine algae ("red tides or toxic blooms") (Cockburn 1971; Schantz 1973; Wood 1976).

Micromorphology

The micromorphology of the Moita bone was analyzed in the plastic-embedded thin sections. Bone preservation was variable even within the same section. There were small randomly scattered clear areas in which normal microstructural features were adjacent to features less well preserved. Haversian systems composed of osteons with their Haversian canals and cement lines were present (Plate 2, page 53). The canals contained no blood vessel remnants or cellular elements. Structures claimed to be blood vessels and red blood cells have occasionally been observed in the Haversian canals of archaeological bone (Graf 1949; Stout and Teitelbaum 1976a). Volkmann canals were also evident in some of the thin sections. Acellular osteocytic lacunae and fragments of previously formed older osteons were also identified. Canaliculi were not observed possibly because of infilling with calcified material or inadequate microscopic magnification.

In the less well preserved areas the collagenous lamellae in the osteons appeared distorted and swollen (Plate 3, page 53) The individual lamellae appeared to be tearing apart or separating from each other and from the underlying framework of thin concentric fibers. The disruption and separation radiated out from the Haversian canals like the spokes of a wheel and appeared

Plate 2. Photomicrograph of a resin-embedded cross section of cortical bone from a human femur (Moita) revealed by light microscopy. A typical osteon (lower right), osteon fragment (OF), Haversian canal (H), cement line (CL), lacunae (L), interstitial lamellae (IL), and Volkmann's canal (V) are labeled. Unstained. X 105.

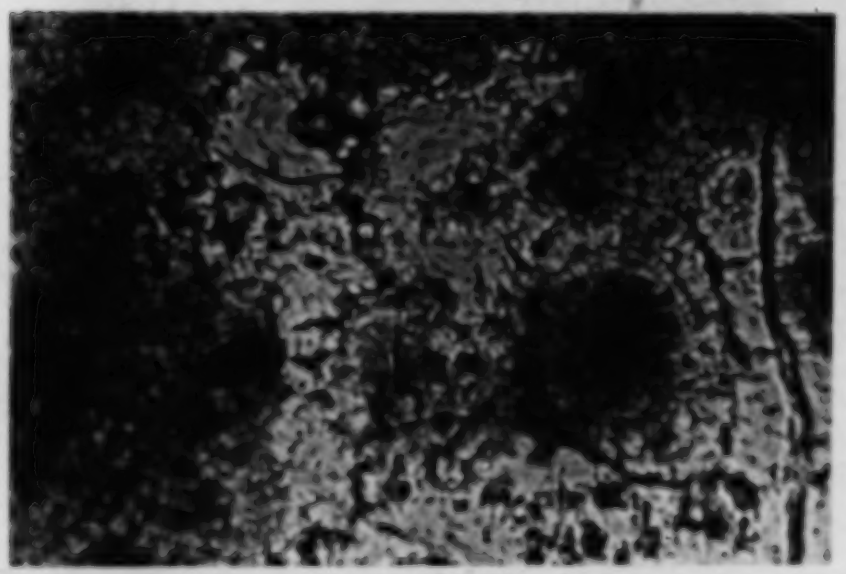

Plate 3. Photomicrograph of a cross section of a human femur illustrating the disruption and distortion of the microstructures in Moita bone. Resin-embedded, unstained. Light microscopy. X 105.

to be lifting away from the deeper concentric fibers. Spaces could be observed between these fibers now that the weaving together was disrupted. The "spokes" appeared to consist of short segments of material, perhaps mineral such as hydroxyapatite, from previously deposited calcium phosphate. This type of disaggregation has also been observed in ancient skeletal remains recovered from burials in Sweden (Graf 1949) and Israel (Salomon and Haas 1967).

The cement lines were often indistinct in these areas apparently because of the discoloration and partly because of poor preservation. There were some osteons that showed signs of fracturing along the cement lines. Large areas of matrix displayed a spongy "cottage cheese-like" appearance without recognizable structures. Even the clearest ~~and best~~ preserved sections had areas that were less well preserved than others.

The concentric rings seen in the osteons of the better preserved areas in the Moita bone appeared to be more highly calcified than the surrounding tissue in the osteons (Plate 4, page 55). In fluorescent light these concentric rings were clearly visible as a light yellowish-green and they appeared to fluoresce against the green color of the adjacent bone tissue (see discussion page 105). Some concentric rings appeared brighter than others in the same section (Plate 5, page 55). The fluorescent light appeared to outline these concentric rings even in the areas most affected by the ash-gray discoloration.

The inner lamellae adjacent to the Haversian canals or reforming cavities were brighter colored than the light yellow-

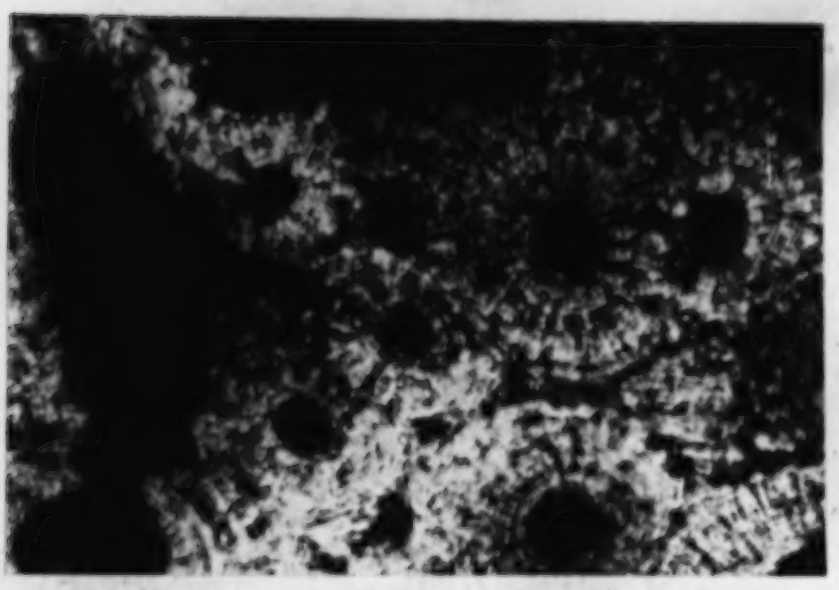

Plate 4. Photomicrograph of a cross section of cortical bone from a human femur (Moita) illustrating apparently dense, highly calcified inner lamellae of Haversian canals. Resin-embedded, unstained, undecalcified. Light microscopy. X 105.

Plate 5. Photomicrograph of an unstained cross section of cortical bone from a human femur (Moita) showing fluorescence on the inner lamellae of Haversian canals and within the osteons. Fluorescence microscopy. X 105.

green of the concentric rings in the outer areas of the osteon. These fluorescent rings were present in all of the thin sections of bone in the sample. Some osteons lacked a ring but most had either a single or a double ring. Triple rings were very infrequent. One thin section had one triple ring while another howed two triple rings. Three thin sections were observed with only a faint indication of a triple ring. The rings varied in thickness from 5-10 um. They were randomly scattered throughout the sections so that the fluorescent rings were not more frequent in any one area than another. The large resorption spaces did not show these types of rings. There were few indications of cement lines with this technique.

The bone had diffuse areas of brighter yellow-green, sometimes near the periosteal border, but on others it was nearer the endosteal regions. The areas most affected by the ash-gray stain did not show more yellow-green coloration than the less obscured areas.

The mounting media, Permount and Diatex, and Ward's Bio-Plastic embedding medium were tested for fluorescence with negative results. When a small section of the Moita bone was examined in distilled water it showed apparent fluorescence. This result indicates that the fluorescence emanated from the bone rather than the mounting or embedding material.

Straight lines, transverse to the long axis of the femur, were observed in the thin sections with both standard light microscopy and fluorescent techniques. When these lines were viewed under ordinary light microscopy they were easily

distinguished from the adjacent bone. They appeared to reflect more light and appeared denser suggesting, perhaps, a higher degree of calcification (Plate 6, page 58). In fluorescent light the lines showed fluorescence comparable to the concentric rings in the inner lamellae of the osteons. Their lighter yellow-green color contrasted distinctly against the green of the background bone. There were no apparent differences in color intensity (Plate 7, page 58).

The lines were of a consistent thickness measuring 10 um in all sections with only three exceptions. These were finer lines measuring 5 um. The lines varied in length, in number, and in distance apart. Some lines crossed the entire width of the thin section while others were much shorter, presumably, as a result of resorption and /or osteon formation. The longer lines were seldom interrupted by erosion cavities.

There was a noticeable difference in the spacing between the lines. Several sections had sets of three or four closely spaced lines within an area of 110 um - 250 um. One section displayed a broad expanse of lamellar bone, 1500 um wide, bounded by long transverse lines. There were osteocyte lacunae within the boundaries but there were no resorbing or forming cavities until the far end of the thin section. The distance between most lines ranged from 30 um - 900 um.

In the sample of 32 cores there were 12 (37.5%) thin sections showing transverse lines. The number of lines in the thin sections varied in number from 2 to 12. There were 8 males, 3 females, and one individual of unknown sex with these lines.

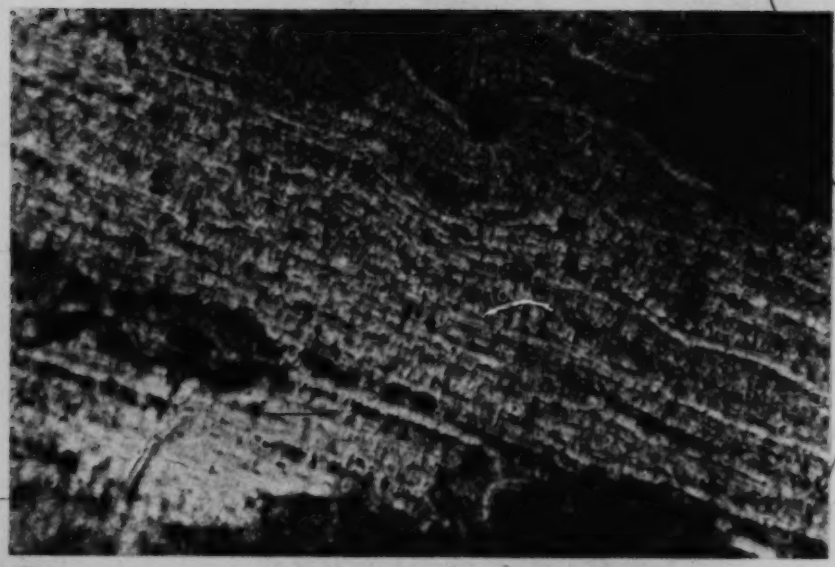

Plate 6. Photomicrograph of an unstained cross section of cortical bone from a human femur (Moita) showing transverse lines revealed by light microscopy. Several small areas of ash-gray discoloration (upper and lower right). Resin-embedded, undecalcified, unstained. X 105.

Plate 7. Same section as Plate 6. Fluorescence microscopy. X 105.

Age Determination

The fact that the ash-gray discoloration was heaviest on the periosteal and endosteal borders prevented observation of the osteonal features required for age-at-death estimates. There were not enough clear areas to permit counting of osteons and their fragments. Ahlqvist and Damsten's method (1969) could not be applied. Their method employs polarized light and a 10 x 10 ocular grid measuring 1 mm^2 at the level of the section, to count the number of osteons and osteon fragments more than half filling each small square in the grid. The percentage of remodeled bone calculated from 4 periosteal fields is entered into a regression formula to estimate age at death.

The mid-cortex was less affected than the outer and inner areas but in many sections the entire thin section was obscured by the discoloration. In the clear areas it was often impossible to determine whether the Haversian canals were in the initial process of resorbing, or in the final stage of reforming, as many of the inner lamellae were partly or completely obscured by the discoloration affecting the bone. At least 90 % of the Haversian canal perimeter must show no evidence of remodeling (Stout and Teitelbaum 1976b). This fact along with the indistinctness of cement lines and the difficulty in discriminating osteon fragments from interstitial lamellae would have undermined the accuracy of age estimates.

Osteonal area determined by point counting also gives accurate age estimates (Thompson 1979) but distinct cement lines are required to assess their areal extent. Thompson's core

technique (1979) was the preferred method as his technique has been developed and tested using cores. Thompson prepares thin sections 80 um thick from cortical bone cores measuring 0.4 cm in diameter. A 10x10 grid measuring 0.992 mm^2 (at x100) is employed in the stereological procedure. The total area, number, and perimeter of osteons and Haversian canals are quantified in four adjacent periosteal fields by a point counting method. These values are used in a regression formula to obtain an estimate of age at death. This method, however, could not be used because of several limitations: there were very few clear areas to assess in the Moita sections; the cement lines were indistinct; the discoloration was too widespread; preservation was poor; and there was no grid available that could be used for point counting.

The expectation that fluorescent light illumination would outline the cement lines to allow age estimates to be made was not realized. The cement lines were not observed possibly because of the lack of preservation or because cement lines contain no collagen, even in fresh bone (Bloom and Fawcett 1975; Ascenzi 1983; Parfitt 1983), and therefore might not be visualized in fluorescent light.

These results were particularly disappointing since age at death is essential for palaeodemographic purposes (Angel 1969; Ubelaker 1974). Trends in length of life and mortality patterns based on this group of individuals at Moita must therefore be determined from other age indicators. It has been suggested that using a combination of several methods for age determination

provides more reliable data than depending on a single technique (Singh and Gunberg 1970; Acsadi and Nemeskeri 1970; Weinstein et al. 1981; Meindl et al. 1983).

In view of the results of this research it appears that aging techniques based on osteon counts are not always applicable and cannot be relied upon to provide age estimates for all ancient skeletal material. Human remains recovered from shell middens such as the Moita site present particular problems that have to be solved if age assessments are to be obtained. Another researcher also has concluded that histological aging techniques may not be applicable to all archaeological skeletal material (Pfeiffer 1985). The technique of osteon counting for age assessment (Kerley 1965) has been modified (Ahlqvist and Damsten 1969; Singh and Gunberg 1970), revised (Kerley and Ubelaker 1978), and compared (Bouvier and Ubelaker 1977; Stout and Gehlert 1980).

These and other problems in the use of histological aging methods have been discussed (Stout and Gehlert 1980; Kurzawski 1983; Lazenby 1984). Differences in the definition of an osteon, in grid size, field of view and number of fields assessed are several of the problems that have been discussed. Lack of confidence in the ability of histological techniques to provide accurate age estimates (Bocquet-Appel and Masset 1982; Lazenby 1984) has led Lazenby to suggest that researchers discontinue using these methods for the present. Dissenting opinions contend that histological techniques do/can accurately predict age (Laughlin et al. 1979; Buikstra and Konigsberg 1985). It appears

that problems with the existing techniques need to be resolved and the methods refined and standardized before they can be used with confidence.

The Porosity Counting Method

In an attempt to examine the distribution and to quantify the amount of porosity present, resorption spaces measuring between approximately 80 um and 400 um were counted. A lower limit of 80 um was chosen to avoid including Haversian canals in the count. Haversian canals vary in diameter as reported by different researchers: the diameter remains unchanged (Curry 1964) up to 40-50 years of age (Duncan 1976); it increases slightly in diameter with increasing age (Barer and Jowsey 1967); it decreases with age (Singh and Gunberg 1970); it is larger than 100 um (Hall 1978); it has a diameter between 22 and 110 um (Bloom and Fawcett 1975); and it averages approximately 50 um in diameter (Windle 1969; Beddoe 1977; Richman et al. 1979; Ortner and Putschar 1981). Type II resorption cavities (a dilation in the walls of Haversian canals of well mineralized osteons) have a diameter 10 - 20 um larger than those of Haversian canals (Richman et al. 1979 :209). While the major amount of porosity in cortical bone is derived from the resorption spaces, there is a small contribution of $\pm$ 15% made by Haversian canals, Volkmann's canals and osteocytic lacunae (Duncan 1976).

An upper limit of 400 um was chosen because osteons have a diameter of approximately 300 um (Ortner and Putschar 1981; Mathews 1980; although some may reach a maximum of only 200 um

in diameter (Parfitt 1983). An osteon provides evidence of the former size of a resorption cavity eroded by osteoclasts (Ogden 1980) and its subsequent refill by bone building osteoblasts. It was suspected that resorption cavities larger than 400 um may have resulted from the sawing process, from post-mortem damage or that several smaller bone-spaces may have merged to form the large resorption cavities.

Porosity

The inability to obtain age estimates with osteon counts (see discussion page 59) encouraged a search for alternative indicators that might provide a general impression of aging. Aging is accompanied, in humans at least, by bone loss. The changes observed in cortical porosity, density and thickness are thought to be associated with the aging process and health status.

Since cortical bone resorption has been reported to increase from the cortical endosteal areas to the periosteum with increasing age (Atkinson 1964), and especially in post-menopausal or senile osteoporosis (Frost 1973), it has been suggested that the entire cortical thickness be included in all measurements of bone loss (Atkinson 1964).

Resorption spaces in fields measuring 0.92 mm^2 were counted across each thin section of Moita bone under x100 magnification. Three lines of counts were made; one at the periosteal border, one in the mid-cortex and one along the endosteal margin. Between four and nine fields, measuring 0.92 mm^2, were observable across

the thin sections. Two counts were made by the same analyst but were separated by a time interval of thirty days. In consultation with Jackes, the results were analyzed for the mean number of resorption spaces per each line of count.

When the three cortical areas were compared in the second count there was a slightly greater number of resorption spaces or cavities in the periosteal one-third (Table 3, page 150). In 19 of the 32 (59%) individuals the outer periosteal one-third contained a slightly greater percentage of the resorption bone cavities than either the mid-cortex or the endosteal one-third. This was observed in both males and females. One adolescent male and one female showed 33% more resorption spaces in the periosteal one-third in comparison with the endosteal one-third. However, comparison of the two individuals with the highest (L57) and the lowest (L35) mean number of periosteal resorption spaces revealed no significant difference based on chi square, whether all three areas, (periosteal, mid-cortex, endosteal), were compared or just the periosteal and endosteal areas (Table 4). The difference was non-significant in spite of the fact that L57 had 51% of the resorption spaces in the outer periosteal one-third while L35 had only 29% of the resorption cavities in the outer periosteal one-third.

The mean number of resorption spaces for each individual was expressed as the mean score of the three counts across the three cortical areas, the periosteal one-third (P), the mid-cortical one-third (M), and the endosteal one-third (E). As an example, the data obtained from a count of the three areas in count two on

L57 is displayed in Table 5 (page 151).

The mean number of resorption spaces used for the analysis of L57 was 11.35; the means from each one-third were added together and divided by three to obtain the total cortical area means (the means of the means), rather than 11.39 (see Table 5).

The distribution of these total cortical area means (for both counts one and two) was bell-shaped indicating no association with age. No linear trends in age were obtained using the means of the means for overall bone porosity across the three cortical areas. This suggests that population variation was sampled. Individuals falling beyond $\pm$ 2 sd of the total population mean for the number of resorption spaces were compared using chi square. No significant differences were found between individuals lying at the upper and lower ends of the bell-shaped distribution.

In spite of the fact that the mean of the total means was 10 in both counts (one and two) of the three cortical areas (PME) the correlations between counts one and two were not perfect (Table 6a, page 152).

In addition, the correlations among the three cortical areas (PME) differed between the two counts and were generally low as shown by the correlation matrix (Table 6b, page 152).

A short summary of the results up to this point is given as folows. There was no difference between individuals with a high or low mean number of periosteal resorption spaces on the basis of the distribution among the three cortical areas (Table 4). Correlations among the three cortical areas were low in count one

(0.610 for P and M) and no correlation was found among the three cortical areas in count two (Table 6b). The correlation between count one and count two (Table 6a) was highest (r = 0.880) for the endosteal one-third.

Because count one and count two for the endosteal one-third revealed a high correlation and thus perhaps greater repeatability it was decided to concentrate on the data from this region in further analysis. This decision is justified since cortical width is decreased with increasing age due to endosteal bone loss (Frost 1973; Albanese 1977). A separate and third count was taken on the endosteal one-third in order to confirm repeatability. The correlation of count three with count one (r = 0.766) and with count two (r = 0.924) indicates increasing accuracy and justifies the assumption of repeatability. Four fields spaced apart as equally as possible were assessed in the endosteal one-third. Each field measured 0.92 mm^2 at the level of the section. Microscopic magnification of x100 was utilized in counting resorption spaces measuring between 80 um and 400 um. The total count for the four fields was divided by four to obtain the mean number of resorption spaces (porosity) per 0.92 mm^2 field. Data from endosteal count three of porosity was used for subsequent analyses.

The number of resorption cavities in endosteal counts two and three were checked with the Singh Index to determine whether there was a relationship between endosteal porosity in cortical bone and trabecular changes in the proximal femur. The Singh Index (Singh et al. 1970; Singh et al. 1972) is a grading system

based on changes occurring in the trabecular bone of the femoral head and neck with increasing age and osteoporosis. The cancellous bone in the femoral head and neck consists of four groups of trabeculae arranged into distinct patterns. The arcs formed by the principal and secondary compressive groups originate from the inner (medial) cortex of the femoral shaft while the arcs formed by the principal and secondary tensile groups arise from the outer (lateral) cortical bone of the femoral shaft (Figure 4, page 68). Changes in the trabecular pattern can be observed in radiographs of the proximal end of the femur. As the arcs formed by the compressive and tensile trabeculae decrease in number and thickness the Singh Index decreases in value.

The index ranges from a grading of six in normal individuals to a low value of one with increasing age and severe osteoporotic changes. A grade of three and lower indicates osteoporotic individuals. At this stage, the principal tensile trabeculae have decreased in number and the arcs formed by the trabeculae have been interrupted by resorption. Secondary trabeculae of both the tensile and compressive groups have been resorbed. The principal compressive trabeculae are still clearly visible.

The femoral radiographs were independently analyzed by two individuals (Jackes and Palmer) and all differences in grading were reassessed. The head and neck of twenty femora were sufficiently complete to permit evaluation for the Singh Index. These twenty femora were from the same group that provided cores for analysis.

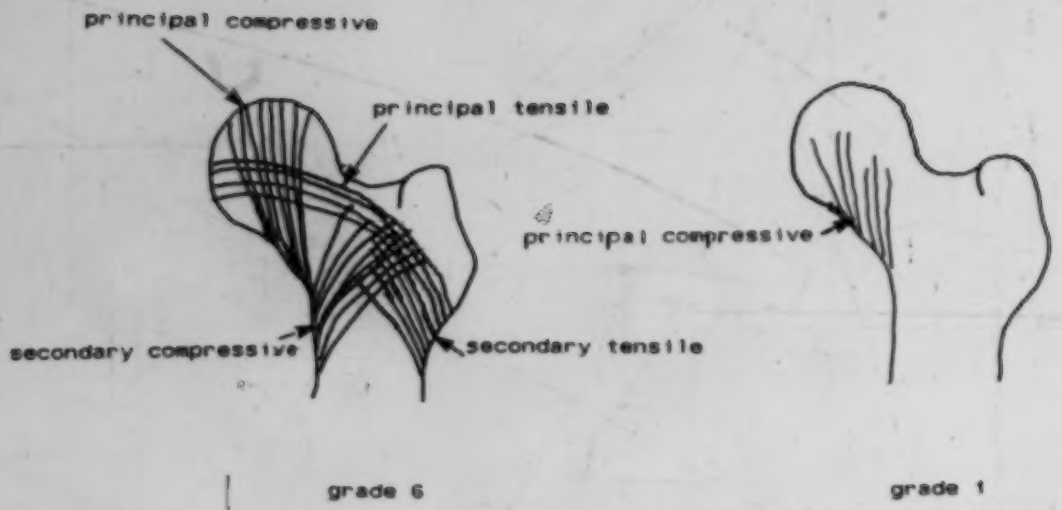

Figure 4. Diagram of the trabecular pattern in the head of the femur. Grade 6: normal trabecular pattern. Grade 1: osteoporotic, few trabeculae visible.

The cortico-endosteal porosity across the width of the section in total count two and the Singh Index showed some relationship ($r = -0.475$, $P = > 0.05$, $n = 16$). A very slight increase in correlation ($r = -0.493$, $P = < 0.05$, $n = 18$) was obtained between the Singh Index and endosteal count three. There was a tendency for the Index to decrease as the number of resorption spaces increased. At the osteoporotic level of three on the Singh Index the mean number of endosteal resorption spaces per 0.92 mm^2 was 11.25 and 11.50 compared to a total mean of 9.35 spaces per 0.92 mm^2. The male mean ($\bar{x} = 9.28$, $n = 19$) was only slightly lower than the mean for females ($\bar{x} = 9.50$, $n = 12$).

The number of resorption spaces differed between opposite sides of each thin section. Unfortunately only the central upper edge of the proximal periosteum was marked on the cores. The section sides were not marked so it became impossible to determine whether these differences were consistent for medial or lateral sides or whether it was just an example of random variation.

Marked differences were observed between the two sides of six thin sections. The differences ranged from a value of 11 to 15. One thin section, from a female (L1), had 42 empty bone spaces on one side of the section and 27 on the other side.

Two individuals, one male (L18) and one female (R20), were represented by both right and left femora. The female thin section had a difference of 11 spaces between sides in the section from the left femur; the right femoral section showed only a small variation in the number of spaces. The male

individual showed little difference between the section sides of either femur.

Counts revealed a higher mean number of resorption spaces recorded in the endosteal region of the right femora for both sexes (L18, R20). These same two individuals also had a higher mean number of resorption spaces in the right femora when the means of the three areas (PME) in the entire thin section were tabulated in count two. The mean number of spaces was greater in the female section (13.22) compared to the number (10.71) for the male.

Transverse lines in the thin sections of these same two individuals may provide another indication of differences between right and left sides. The left femoral sections had a greater number of lines than were observed in the right but these lines were more frequent in the section from the female.

The ages discussed in the following paragraphs were obtained from Jackes' preliminary age estimates based on attrition grades (Jackes 1984). The attrition ages are based on a seriation of all Moita mandibles (n = 50) aged fifteen years and over. The seriation involved the ordering of mandibles based on the wear of lower molars from least wear to greatest. Each molar was assessed on nine attrition levels from no occlusal wear (0) to no occlusal enamel remaining (8). The mandibles were then grouped on the basis of attrition patterns among the three molars into eleven overall attrition grades, taking anterior tooth wear into account. The eleven attrition grades are here arbitrarily given equal five year age ranges from 15-70 years (Jackes 1984).

Figure 5 (page 72), illustrating the number of endosteal bone spaces in both sexes according to 5 year age groups, shows an uneven pattern. Males show a sharp rise in the number of resorption spaces from 15 to 20 years of age with peaks at 20-25 and 35-40. After age 50 there is a steady rise in the number of resorption spaces. The earliest age for females in the sample is 20-25 years. A sharp rise in the number of resorption spaces from age 20-30 is followed by a slight downward slope to age 45. One female, aged 55 to 60, provides the only value obtained for the number of resorption spaces after age 45. The line depicting the number of resorption spaces in females lies above that shown for males except after approximately age 50 when males have a higher number of resorption spaces than is shown by the downward slope of the lines for females.

Cortical Thickness

The cortical thickness of the male sample ranged from 4.595 mm to 8.020 mm while the female sample ranged from 5.131 to 7.452 mm. The male with the low score had a cortical thickness that was lower than the female minimum. The mean cortical thickness for males (6.409 mm, sd 0.92, n = 20) and females (5.941 mm, sd 0.72, n = 12) differed by 0.555 mm. Student's t was used to test the significance of this difference, which was proved to be non-significant at the 0.10 level ($t = 1.84$, $df = 31$, $P = 0.10$).

Age groupings based on attrition grades (Figure 6, page 73) showed that male cortical thickness was greatest between the ages of 35-40 years. There was not a steady increase in thickness as

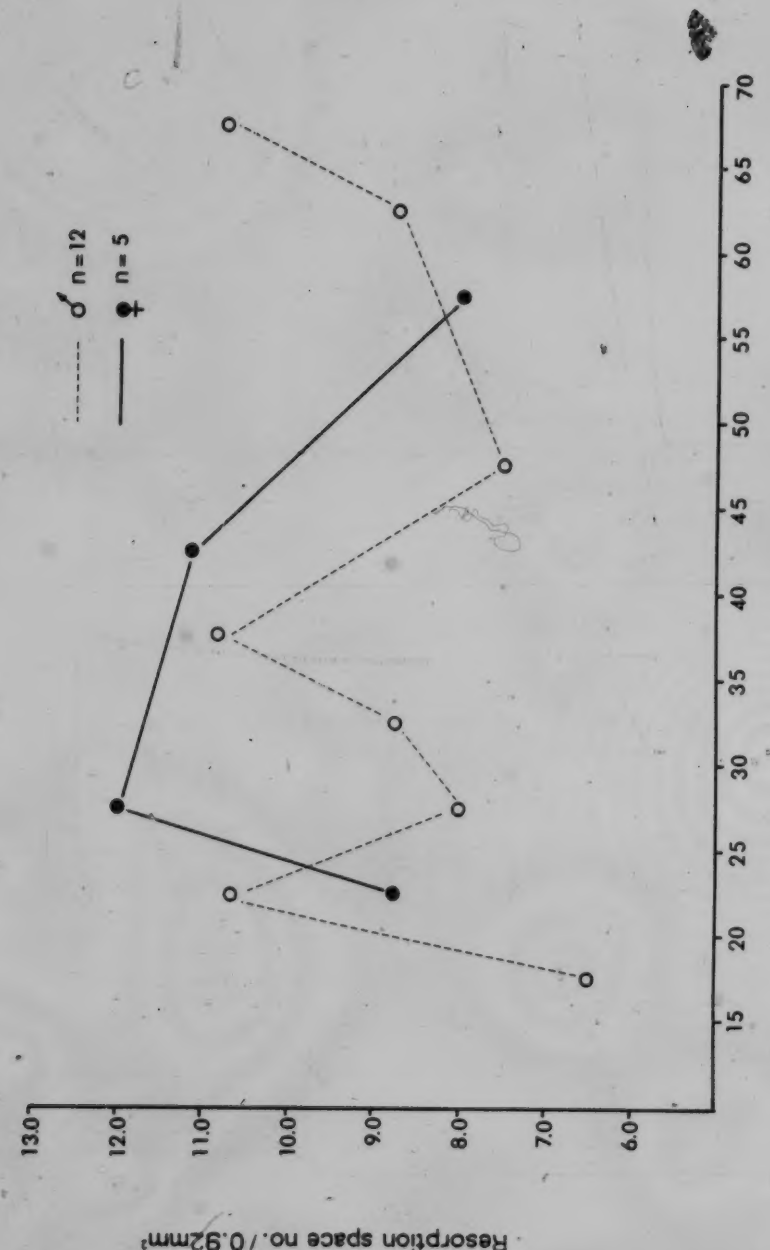

Figure 5. Cortical resorption vs age (by attrition grades). Males and females.

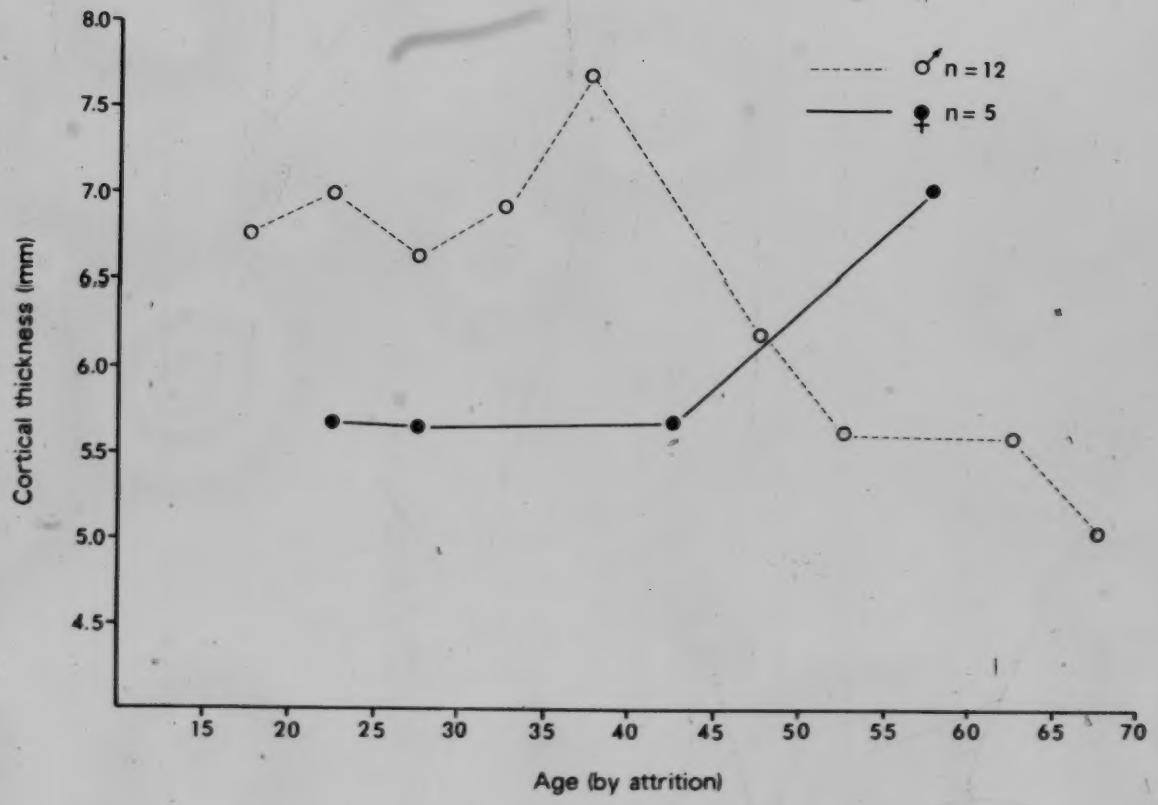

Figure 6. Cortical thickness (mm) vs age (by attrition grades). Males and females.

shown by the drop between the ages of 25-30. After age 30 there was a steady rise in cortical thickness to the age of 35-40 followed by a fairly steep and progressive decline into older age. The increase in cortical thickness in females followed a different pattern. There was a slight decrease in the 20-30 year age group followed by a slight increase to age 40. After the age of 40-45 there was a sharp rise in cortical thickness into middle age. Ages younger than 20 or older than 60 were not represented among the female sample.

Cortical thickness for samples of pooled sexes was not significantly associated with density ($r = -0.008$, $n = 33$), endosteal count three ($r = -0.044$, $n = 32$), or with the Singh Index ($r = 0.166$, $n = 19$). Cortical thickness and ages based on attrition grades showed a low negative correlation ($r = -0.315$, $P = > 0.10$, $n = 21$).

Density

Density values obtained from the bone cores of both sexes ranged from 1.21 g/cm^3 to 2.94 g/cm^3 with a mean of 2.07 g/cm^3, ($sd = 0.45$, $n = 33$). Separate values for males ($\bar{x} = 2.12$ g/cm^3, $sd = 0.43$, $n = 20$) and females ($\bar{x} = 2.05$ g/cm^3, $sd = 0.42$, $n = 12$) show that the mean density values do not vary greatly from the total mean. Females have a slightly lower spread of density scores ranging between 1.63 g/cm^3 to 2.94 g/cm^3 compared to the scores of 1.50 g/cm^3 to 2.92 g/cm^3 for males. The actual weight of the cores ranged from 0.2 to 0.5 grams.

Figure 7 (page 75) shows that density is lowest in females

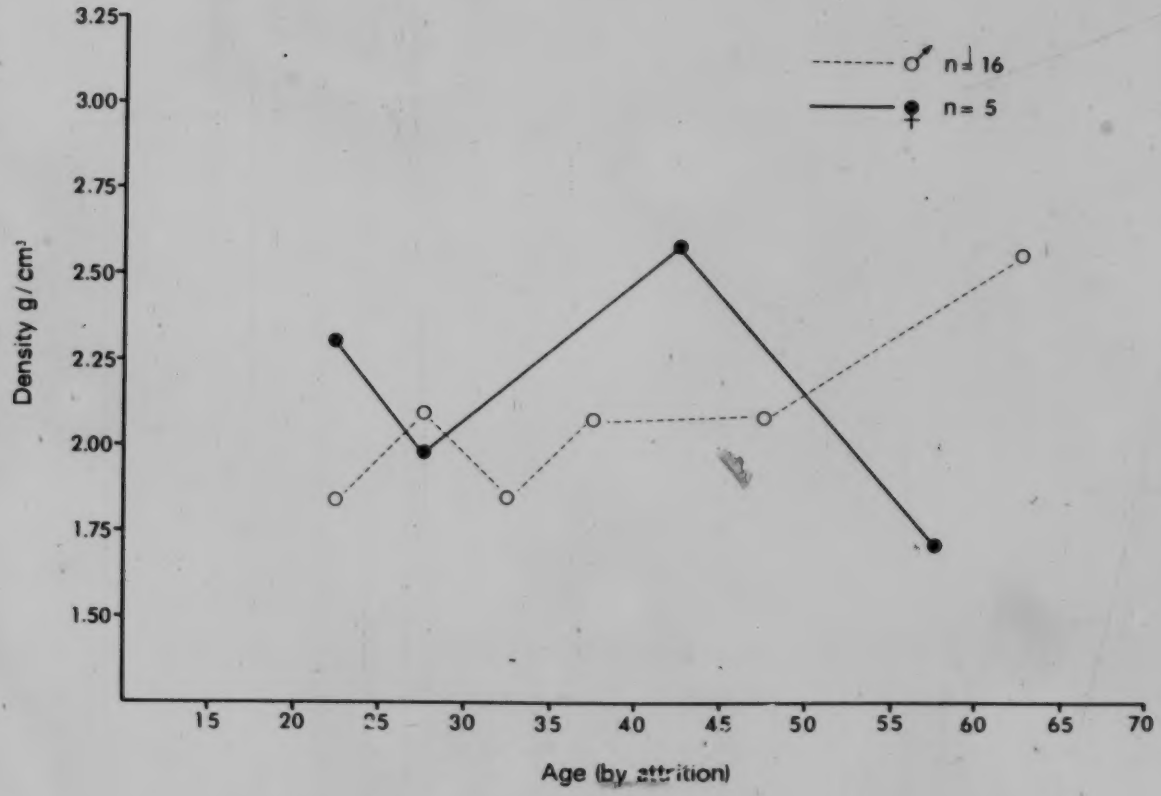

Figure 7. Cortical density g/cm³ vs age (by attrition grades). Males and females.

at the ages of 25-30 with values rising to their highest levels at 40-45 years of age followed by a rapid decrease into older ages. Males, in contrast to females, generally show an increase in density from the lowest levels at 20-25 years of age to the ages of 60-65. After age 65 density declines in males.

Cortical density and Singh's Index showed a moderate negative correlation ($r = -0.488$, $P = < 0.05$, $n = 19$) suggesting that density increases with age in the Moita sample. Low correlations were obtained between density and endosteal spaces in count three ($r = 0.399$, $P = < 0.05$, $n = 32$) and between density and attrition grades ($r = 0.238$, $P = > 0.10$, $n = 17$). Density and cortical thickness showed no association ($r = -0.008$).

In spite of the low correlations, the contrast between the two sexes in relation to endosteal resorption spaces, cortical thickness and density are readily apparent in Figures 5, 6, and 7 when attrition ages are used in the comparisons. In females, an increase in the number of endosteal resorption spaces tends to coincide with a decrease in cortical thickness ($r = -0.670$, $P = > 0.10$, $n = 5$), however, the correlations might change with a larger sample. Females have a higher number of resorption spaces to age 55 and higher values for density than males at ages 20-25 and 40-45. At approximately 50 years of age a reversal in resorption scores occurs between the sexes. This reversal also occurs in cortical thickness at about 50 years of age when cortical thickness increases for females but decreases for males. As shown in Figures 5 and 6, cortical thickness and endosteal resorption

spaces ($r = 0.308$, $P = > 0.10$, $n = 12$) in males tend to rise or fall together until after age 50 when the pattern changes; resorption spaces increase in number along with a drop in thickness. All three figures show the male-female lines intersecting and then diverging where value levels are reversed between the sexes.

A negative correlation was obtained between the Singh Index and density ($r = - 0.488$, $P = < 0.05$, $n = 19$) and between the Singh Index and endosteal resorption spaces in both counts two ($n = 16$, $r = -0.475$, $P = > 0.05$) and three ($n = 18$, $r = -0.493$, $P = < 0.05$). Although there was only a weak correlation the results indicated a tendency for density and porosity to increase as values of the Singh Index declined.

When scores were seriated from low to high a few of the same individuals clustered together at either end of the density and endosteal porosity values. For instance, the youngest definitely aged individual (L57) had low density scores, a low number of resorption spaces and a high Singh Index value (see Table 1, page 148). At the other extreme, three individuals had high density levels, a high number of resorption spaces and a low grading on the Singh Index. The other individuals in the sample showed variable results with no observable pattern. The cortical widths did not seriate in association with density and porosity.

Although it might be possible to separate several of the younger individuals from the older individuals on the basis of porosity and density, the reliability of such a separation may be

CHAPTER 1V

DISCUSSION

Review Of Bone Function, Growth, Morphology , And Disease

The human skeleton consists of bone, a hard calcified material that maintains the shape of the physical body while supporting and protecting the soft tissues within the body. It acts as a metabolic storehouse for calcium and phosphorus and it plays a major role in the regulation of homeostatic serum calcium levels. Calcium and phosphorus can be withdrawn from bone into the circulation for use in repair, fetal growth, lactation and during times of mineral deprivation (Hancox 1972; Hall 1978).

Bone is a living tissue in a constant state of change. It undergoes alternating periods of resorption and formation throughout the lifespan of the individual (Frost 1964; Laeroix 1971). This process constitutes the remodeling system and it is carried out by bone cells, the bone-forming osteoblasts and the bone-resorbing osteoclasts. All bone remodeling occurs on either the periosteal, intracortical (Haversian) or cortical-endosteal bone surfaces. Remodeling and distribution of bone loss between these surfaces varies with age and nutrition, during growth and in many pathological conditions.

Resorption of bone by osteoclasts and formation by osteoblasts on the periosteal surface results in the slow outward expansion of a bone such as the femur throughout adult life, with formation exceeding resorption on the periosteal surface, but the

in childhood (Frost 1973). Periosteal apposition or the addition of layers of compact bone on the periosteal surface is seen histologically as circumferential lamellar bone. Lifelong subperiosteal apposition of long bones is accompanied by enlargement of the medullary cavity (Smith and Walker 1964; Garn 1970; Ruff and Hayes 1982). During the growing period the enlarging medullary cavity prevents the formation of heavy bones (Simkiss 1975).

There are two kinds of skeletal bone, spongy and compact (Bloom and Fawcett 1975; Parfitt 1983). The ends of long bones such as the femur consist of spongy bone covered by a thin layer of compact bone while the walls of the hollow shaft are composed of thick layers of compact bone. Compact bone consists of mineralized bone matrix deposited in concentric lamellae or layers around a central vascular canal to form an osteon or Haversian system. The central canals of Haversian systems run longitudinally and parallel to the long axis of the femur. An osteon represents a previously resorbed area of bone that has subsequently been refilled with bone. Haversian canals are connected to each other and to other blood vessels by Volkmann's canals. A cement line marks the outer limit of each osteon.

Osteoblasts form the organic matrix (osteoid) composed of collagen and mucopolysaccharides (glycoprotein). Inorganic bone mineral consisting of calcium, phosphate and hydroxyl ions is deposited on the collagen fibers in the organic matrix. During the process of mineralization the minerals are converted to

bone (Jowsey and Gorden 1971).

During matrix formation osteoblasts are frequently surrounded by matrix and become trapped in small bone spaces called lacunae (Bloom and Fawcett 1975). The entrapped osteoblasts are termed osteocytes. Their cytoplasmic processes are connected to a blood supply and to the processes of other osteocytes via minute canaliculi. Canaliculi are slender branching tube-like channels radiating out into the bone from each lacuna but the canaliculi do not cross the cement lines holding adjacent osteons together. These interconnections presumably provide rapid transport for the exchange of nutrients and minerals between the cells and the nearest vascular connection (Bloom and Fawcett 1975).

The responses made by bone cells to changes in metabolic, nutritional, or endocrine levels is written as a semi-permanent record in the microstructure of bone (Frost 1964; Sissons 1971). Since these bone patterns are the result of life processes, but last for longer than the life of the individual, the patterns recorded in bone may be useful in analyzing archaeological bone for evidence of nutritional stress, pathology and bone loss (Martin 1981; Ortner and Putschar 1981).

The loss of bone in the later years of life is a universal problem affecting all races and both sexes but it occurs to a lesser degree in males than females (Nordin et al. 1970; Albanese 1977; Jaworski 1983). Bone loss is not a recent phenomenon restricted to living populations; it has been observed in fossil

"Our fossil ancestors lost bone if they lived...." (Garn 1981:3)

The cause of bone loss with increasing age has been attributed to a variety of factors. Low dietary calcium intake, a decrease in calcium absorption across the intestinal wall, vitamin D deficient states, protein-calorie malnutrition, decreasing levels of sex hormones, and inactivity have been implicated by various researchers at different times. In spite of much research effort over the years a specific cause of bone loss has not as yet been isolated. It is possible that age-related bone loss may be a combination of several of these factors.

Osteoporosis is the term that is used to describe age-related bone loss (Frost 1973). Although a decrease in bone mass is a typical feature of osteoporosis there are individual differences in the rates and the amount of bone that is lost (Johnston et al. 1980). Senile and post-menopausal osteoporosis is characterized by excessive endosteal resorption (Martin 1981; Thompson et al. 1981). As a consequence there is a decrease in cortical thickness. Cortical width normally reaches a maximum thickness between 20-40 years of age in both males and females (Albanese 1977; Mazess 1983) because of subperiosteal apposition but the rate decreases thereafter. Maximum bone density in both sexes occurs at approximately age 35 (Goldsmith 1973).

Based on data obtained in populational surveys and research on groups of normal individuals it appears that most populations experience bone loss with progressive age. Bone loss generally

1977). Dequeker (1972) found a decrease in compact bone mass after age 40 in both males and females in the Netherlands, Nigeria, and Belgium but females had a faster rate of loss. Mineral loss of calcium and a decline in bone density begins after age 45 in females and after age 60 in males based on values of normal U.S. whites (Mazess and Cameron 1973), and after age 50 for both males and females in Japan, India, Finland, and central America (Nordin 1966). A survey of mineral loss from bone in several different ethnic groups in California showed that females experienced a "precipitous decline" after age 45 and that loss occurred after age 65 in males (Goldsmith 1973). An exception has been noted by Adams et al. 1970) who report that 38% of elderly males and 22% of elderly females did not lose bone with increasing age during their longitudinal study on bone loss. Since bone loss in modern populations is a major health problem among the elderly it is of interest to inquire into how prevalent it was in ancestral populations.

Bone loss has been studied by numerous researchers utilizing a variety of techniques in both modern and archaeological populations of all ages. Most bones of the body have been studied in relation to changes in porosity, cortical thickness and density.

Cortical bone remodeling has been studied in tibiae obtained at autopsy from modern populations (Ortner 1975), in archaeological rib from Ledders, a Late Woodland site, (Stout and Teitelbaum 1976b), at midshaft in femora of Alaskan Eskimos and

from Sudanese Nubia (Martin and Armelagos 1979).

Remodeling rates are influenced by diet (Stout and Teitelbaum 1976b; Stout 1978; Richman et al. 1979), nutritional adequacy or stress (Martin 1981; Huss-Ashmore 1981), health or disease (Ortner 1970; Stout and Simmons 1979), biomechanical stress (Ubelaker 1974), genetics (Ericksen 1980; Frank 1981), and sex and aging (Frost 1964; Ortner 1975; Martin and Armelagos 1979).

Bone formation rates provide more than age estimates; they present information or evidence of general skeletal health and nutritional status (Stout and Simmons 1979). An imbalance in the ratio of the number of forming osteons to resorption spaces of approximately 10:1 (Ortner and Putschar 1981) or 9:1 (Ortner 1970) may indicate inadequate nutrition or pathology.

Cortical bone loss with increasing age is the result of a disparity between bone formation and resorption (Dequeker 1972) with resorption exceeding formation (Ortner and Putschar 1981). As aging progresses the increase in resorption is greatest in the endosteal area (Atkinson 1964; Duncan 1976; Thompson et al. 1981).

Cortical thickness has been assessed in the femora of autopsy material (Arnold et al. 1966; Bartley and Arnold 1967), in living subjects (Barnett and Nordin 1960; Smith and Walker 1964), and in archaeological femora (Dewey et al. 1969; Van Gerven et al. 1969; Van Gerven et al. 1970; Armelagos et al. 1972; Van Gerven 1973; Bergot and Bocquet 1976; Ericksen 1976;

[illegible] Thompson et al. 1981; Hatch et [illegible]

Several levels along the femoral shaft have been measured (Carlson et al. 1976; Ruff and Hayes 1982; Ericksen 1982). One study has compared cortical thickness and histological changes in the same location (Martin and Armelagos 1979). Other research has looked at subsistence in relation to cortical bone changes in the rib (Saitta 1981), the radius (Perzigian 1973), the femur (Ericksen 1976; Richman et al.1979), and the femur, metacarpals and lumbar vertebrae (Pfeiffer and King 1983).

Cortical thickness is influenced by age, sex, nutritional stress (Albanese 1977), physical activity (Ruff and Hayes 1982), pathology (Dequeker 1972; Frost 1973; Ortner and Putschar 1981), and population differences (Garn 1970; Ericksen 1976; Thompson and Gunness-Hey 1981).

The changes in density associated with increasing age have been studied in cadaver and autopsy samples of femora (Atkinson et al. 1962; Atkinson and Weatherell 1967; Atkinson and Woodhead 1973; Wall et al. 1979), in archaeological femora (Trotter et al. 1960; Thompson et al. 1981), in the spine (Nordin 1966), the fibula, ulna and radius (Mazess and Jones 1972).

Bone mineral content has been determined in radii, ulnae and femur (Perzigian 1973; Mazess and Mather 1975; Laughlin et al. 1979; Frank 1981). Sigmon (1970) took bone scan readings of bone mineral at several levels along the femoral shaft to determine the distribution of bone mineral.

Density and bone mineral content are sensitive to factors such as age, sex, nutrition, physical activity, pathology, population differences (Mazess and Mather 1975; Albanese 1977),

parity, lactation, and the menopause (Goldsmith 1973).

Porosity

There was a pronounced contrast between the Moita male and female patterns of endosteal bone loss and cortical thinning. Although it is possible that metabolic differences in the utilization of available dietary nutrients may have accounted for the diversity, it is also possible that some variation in dietary resources may have existed. Since the diet included a mixture of both marine and terrestrial animals, females may have placed a greater emphasis than males on the consumption of shell-fish. Females may have included shell-fish in their daily gathering activities and in their diet while males may have ingested a larger proportion of meat obtained from hunting. This suggestion is supported by the observation that this type of coastal resource exploitation is presently practiced by male and female hunter-gathers in Arnhem Land in Northern Australia (Meehan 1982) and in South Africa (Bigalke 1973). If this did occur at Moita, the intake of calcium might have been greater in females than in males and could explain why females did not show a loss in cortical thickness in comparison to males.

An increase in endosteal porosity during the reproductive period of females between 25-30 years of age suggests that calcium intake may sometimes have been inadequate to meet the demands of pregnancy and lactation. As a consequence there may have been a drain on maternal reserves to meet these requirements although there was only a slight decrease in cortical thickness

at that time, perhaps, because of high levels of estrogen and progesterone during pregnancy (Kumar et al. 1980). The subsequent decline in endosteal porosity beginning after age 30 continued but was more noticeable after 45 years of age.

Males also exhibited an increase in endosteal porosity from age 20-25. Unlike females, endosteal porosity in males was marked by an instability with high levels occurring again at ages 35-40 with a smaller increase at 30-35 years of age. The steady rise in endosteal porosity after age 45-50 occurs earlier than has been reported for normal males in modern populations. This variability could be diet-related, indicating periods of food scarcity. However, the effects of a food scarcity would likely be shown more drastically by females in view of their role in reproduction, unless there was some association between male status and a particular food item. Intestinal absorption of calcium may have been more efficient in females than in males. Malabsorption of minerals would create a problem with calcium absorption (Nordin 1973; Albanese 1977), however, it is difficult to imagine that a sex-specific malabsorption syndrome was involved.

It has been suggested that some age and sex-specific differences in cortical remodeling and cortical thinning are a reflection of relative health and an indication of nutritional and/or dietary experience (Buikstra and Cook 1980). An early onset of bone loss in Eskimo males in their 40's, similar to the onset in Eskimo females, has been ascribed to the acidic effect of a high protein meat diet (Mazess and Mather 1974, 1975). An

increase in remodeling rates as a result of low calcium intake such as exists in populations dependent on maize agriculture (Stout 1978; Stout and Simmons 1979) or as in ancient and contemporary Eskimos ingesting a meat diet low in calcium (Richman et al. 1979) has been suggested as a possible cause of bone loss. The high remodeling rates may indicate a secondary hyperparathyroid reaction to low calcium availability (Stout 1978; Stout and Simmons 1979).

It has been pointed out that Aleut populations have lower rates of bone loss than Eskimos despite a similar intake of dietary protein (Laughlin et al. 1979). Conflicting reports on the possible role of dietary protein and phosphorus in bone loss has been noted. High protein intake increases calcium excretion (Mazess and Mather 1974; 1975) and leads to osteoporosis (Anand and Linkswiler 1974). Experimental evidence indicates that urinary calcium either shows no change (Spencer et al. 1979) or is decreased (Nordin et al. 1970) when a high protein diet is combined with a high phosphorus content. A high phosphorus content in the diet increases absorption of calcium (Spencer et al. 1979) while phosphorus deprivation increases urinary calcium excretion (Norman 1980). It appears that most human diets are not deficient in protein and phosphorus (Albanese 1977; Spencer et al. 1979) and contain higher levels of phosphorus than calcium but calcium metabolism is unaffected by the disparity (Irving 1973). Apparently the ideal Ca:P ratio of 1:1 (Guthrie 1975; Runyan 1976; Wing and Brown 1979) is seldom a reality in the diet in many countries (Nordin et al. 1970).

The Moita population also consumed a meat diet presumably high in protein, low in calcium, and high in phosphorus along with shell-fish in the diet. For example, rabbit and deer meat contain high levels of protein and low levels of calcium (Souci 1981). Mussels are low in fat and protein content (Parmalee and Klippel 1974), high in phosphorus and relatively high in calcium compared to red meat (Pennington and Church 1980; Adams and Richardson 1980). In order for the sexual differences in resorption to occur, the two sexes may have placed a different emphasis on dietary items or some additional factor may have been involved.

Males may have experienced physiological or psychological stress. As a consequence of stress there may have been a rise in the level of circulating cortisol (Selye 1956; Guyton 1976). If prolonged stress was a factor in the life of older males it could have led to osteoporosis (DeLuca 1982; Manolagos et al. 1979). The bone loss experienced by males may indicate that males were experiencing more stress or were more sensitive to stress than females. Stress and cortisol lower calcium absorption (Alvioli 1983).

Cortical Thickness

Cortical thickness has been assessed in two ways for the Moita femora. The first method was based on a direct measurement, to three decimal places, of the width of the sample cores (Table 1, page 148) indicating the thickness of the mid-femoral anterior cortex. The cores were measured without

modification, since the trabeculae were removed during the coring process.

Nordin's Index, (Barnett and Nordin 1960) was the second method used to measure cortical thickness. The thickness of the cortical bone at femoral midshaft was evaluated on radiographs and expressed as Nordin's Index (Lubell et al. 1986a). The Nordin's Index data were derived from twenty-eight radiographs of Moita femora. The x-rays were anterior-posterior exposures with the femora positioned to lie flat in order to give maximum clarity to the trabecular patterns in the femoral neck and head for later evaluation of the Singh Index (Table 1, page 148). Nordin's Index was calculated as total midshaft breadth/medullary canal breadth (Table 1, page 148). By the age of 35-40 years cortical thickness in the male sample at Moita had reached its maximum. Once initiated, cortical thinning continued and by age 45-50 years cortical thickness was less than that of the 15-20 year old male. The only fairly stable period occurs during the ages from 50-55 to 60-65 years when the cortex loses very little in thickness, presumably, because periosteal apposition and endosteal resorption are equally balanced. Both endosteal porosity (Figure 5, page 74) and cortical thickness (Figure 6, page 75) in males are characterized by sharp increases and decreases in values throughout the lifespan.

The bone of the female sample was not marked by these fluctuations. There was only a slight decrease in cortical thickness during the early reproductive period at ages 25-30 although it was accompanied by a rise in endosteal porosity. The

increase in cortical thickness along with a decrease in endosteal resorption continued and was particularly noticeable after age 40-45 when bone loss might be expected to appear. Endosteal apposition in later adolescence and during pregnancy may be an adaptation for the stress of later lactation (Garn 1970; 1981) and it may account for the increase in cortical thickness. The increase in cortical thickness may indicate an accumulation of bone resulting from several pregnancies. Calcium absorption by the intestine is increased during pregnancy and leads to an increase in cortical thickness (Kumar et al. 1980). As a consequence of multiparity there is more bone in later life especially if lactation is limited or non-existent (Garn 1970; 1981). The accumulation of bone in females after age 25-30 may have occurred if lactation was curtailed by fetal loss, or shortened by early infant death or the practice of infanticide.

Males lost bone but females did not. Females may not have lived long enough after menopausal changes for bone degeneration or osteoporosis to become apparent (Figure 6, page 75). It is also possible that the menopause may have occurred somewhat later than it generally does in modern populations although this cannot be verified. A decrease in bone density of females after age 40 may be more sensitive in predicting later bone loss before it actually affects cortical thickness.

In contrast to most research reports (Bartley and Arnold 1967; Carlson et al. 1976) the males in the Moita sample showed a relatively greater decrease in cortical bone width than the females. After age 45-50 cortical width in males was much less

than that of females. The mean cortical thickness for males was 6.564 mm and 5.208 mm for the 15-40 and the 45-70 year age groups respectively. This represents a 1.356 mm loss or a 20.7% reduction in cortical width with age. A loss this drastic may indicate severe physiological stress. Because of the small number of females in the sample (n = 5) it is difficult to judge the reliability of these results. A further problem is that both males and females in the sample may have been assigned to an incorrect age group.

The age estimates based on the degree of dental attrition may reflect dietary differences. A diet consisting of shell-fish (and sandy grit ?) may have produced a different pattern of dental wear in comparison to one of terrestrial animals. The inclusion of plant foods such as nuts or seeds in the diet may have increased dental attrition depending on their degree of hardness and the quantity consumed.

Other researchers on archaeological samples have calculated the average of several measurements at midshaft and have reported non-significant mean bone loss in males aged 40-50+ at 3.9% in X-group Nubians (Martin et al. 1981), 11.3% at the Campbell site Missouri (Van Gerven et al. 1970), 5.4% at a site in Utah (Van Gerven 1973), and 8.1% at the Campbell site Missouri (Carlson et al. 1976). The 20.7% loss in anterior cortical thickness of Moita males appears to be comparable to the significant 17.74% reduction in the anterior cortex of Arikara Amerindian males (Ericksen 1976), a sedentary group combining horticulture with bison hunting. In all of these cases the females in the sample

lost a significantly greater amount of cortical bone in comparison to males.

These data of diminished cortical thickness in females are in direct contrast to the results obtained for females at Moita, in which there appears to be a reversal in the values obtained for cortical bone loss in males and females. Based on Nordin's Index, the Moita females have a mean of 2.06% greater cortical bone than the males. Female cortical thickness was increased from a mean value of 5.371 mm in the age groups 20-30 to 5.93 mm in those aged between 40-60 years indicating a gain of 0.224 mm or 4.2% in cortical thickness. Since the cortical width was greater in males than females until this age the loss of width appears quite drastic. There has been some suggestion that the rate of bone loss is proportional to the initial mass (Johnston et al. 1979).

The sharp decrease in cortical thickness and the increase in endosteal resorption without an accompanying decrease in bone density in males after age 35-40 may indicate the onset of osteoporosis and relatively early aging compared to present day standards. However, bone density is a function of weight and volume in this research. The weight may be higher because of an increased uptake of external minerals or deposition into some of the resorption spaces or Haversian canals.

Osteoporosis is characterized by less bone than some standard of normal but what remains has normal mineralization (Alvioli 1983) while premature bone loss appears to be associated with a decrease in mineralization (Martin et al. 1981). Secondary

osteoporosis, unrelated to the senile or post-menopausal variety, results from a greater than normal increase in bone resorption in endocrine disease or disordered bone metabolism caused by exogenous factors such as the use of drugs (Alvioli 1983). Clinical studies on normal males indicates that bone loss does not begin until 50-60 years of age, the average age for bone changes in most males (Garn 1970; Irving 1973). Several factors may have been involved in the rapid loss of cortical width in the Moita males. Imbalances in the dietary intake of nutrients such as protein, calcium, or the ingestion of tetracycline, might have created problems.

Endosteal resorption and loss of cortical thickness in adults also occurs in protein-calorie malnutrition (Garn 1972; Huss-Ashmore 1981). Although shell-fish are low in protein and fat (Parmalee and Klippel 1974) it is expected that sufficient protein was obtained from terrestrial animals especially since meat from wild animals contains more protein than fat (Eaton and Konner 1985). An increased intake of protein might account for cortical thinning in males at Moita and lend support to the suggestion of dietary differences between males and females.

It has been noted that alcoholic intake is associated with generalized osteoporosis in modern males (Albanese 1977). A loss of bone mass (Saville 1965), even in relatively young males (Spencer et al. 1979), has been observed with the consumption of alcohol. One report indicates that tetracycline induces urinary calcium loss (Spencer et al. 1979) but the long term effects on bone are unknown. Alcohol consumption and the antibiotic

tetracycline (discussed later in further detail) could have promoted calcium loss in males at Moita. If the males at Moita had consumed alcohol it is possible that reductions in the dietary intake of protein and calcium frequently occurred and were inadequate to maintain cortical bone thickness. A lower intake of calcium, in general, may have occurred if males considered shell-fish as a lower status food and preferred the flesh of wild animals. A loss of calcium from alcohol or tetracycline and a lower calcium intake may have combined to produce thinner cortices in males.

Ortner's research (1970) on modern males suggests that there is a lower osteon formation rate and increased resorption with increasing age and alcoholic intake compared to the rate in normal individuals. These results do not explain the continued rise in density in males after 35-40 years of age especially with increasing endosteal porosity and declining cortical thickness but as previously discussed, postmortem mineral deposition may have resulted in higher values for density. One study has reported a positive correlation between density and age in a small sample of modern bone from Black males (Baker and Angel 1965). The production of alcohol from cultivated grains gathered and stored in mud bins has been suggested as a possible source of contact with tetracycline and alcohol in a sample of the Nubian population (Bassett et al. 1980; Bassett 1981; Hummert and Van Gervin 1982).

The Moita population also show evidence of contact with tetracycline. However, unlike the Nubians, they were not

agriculturists. There has been no evidence reported to indicate the presence of prehistoric grain at the Moita site. The purpose of the excavations in 1880 and in the 1950s may not have included pollen analysis or else soil conditions were not conducive to pollen preservation. According to Henfrey in 1852, the vegetation and its distribution in Portugal had not been extensively studied, although, he does mention stone pine (Pinus pinea) north of the Tagus and both stone pine and oat-grass (Arrhenatherum) in southern Spain..

Several small-grain oat varieties grow in south and west Portugal and in southern Spain. These and barley (Hordeum) are considered as weeds since they grow not only among the sand and rocks near the sea but are found in waste ground and grassland (Tutin et al. 1980). It is not known if these grains are of ancestral origin or if they were present when the Moita people were living at the site.

If the fluorescent markers in the bone were the result of tetracycline-infested grain it may suggest that the grain was used for an alcoholic beverage or as a dietary resource. Since the Moita females, in comparison to males, show no loss of cortical bone mass, yet both sexes show these tetracycline labels in the bone, (see below) it may indicate that males consumed the alcohol while females used only the grain. The grain may have been cooked as porridge or made into a bread. The calcium-binding effects of phytates in the grain (Irving 1973; Albanese 1977) may have reduced any tendency to calcium deficiency, or the calcium content in the shell-fish diet may have been

sufficient for both binding and absorption. It is possible that cooking may have destroyed the action of phytates in the grain (Martin et al. 1981; Huss-Ashmore et al. 1982) or perhaps females did not ingest the grain on a daily basis. Whatever the reason may be , Moita females do not show signs of cortical bone loss.

Density

The highest levels of bone density in the male population are found in the 60-65 year age group. This may reflect the many years of hunting activity experienced by the male. In fact, the major stimulus in maintaining optimal bone density may be regular or habitual physical activity (Jette 1976). Other workers have noted similar increases in bone density with physical activity (Nilsson and Westlin 1971; Montoye et al. 1976).

The rapid rise in bone density from the low values shown by the younger males of 15-20 years to the levels of the 25-30 year old group may coincide with the initial increase in physical activity associated with the hunting way of life. Customary participation in serious hunting activity is not expected until approximately 20 years of age in some contemporary hunter-gatherer cultures. For instance, the !Kung male does not actively engage in productive pursuit of food animals until after marriage between 20-25 years of age (Plog et al. 1980).

The most productive !Kung hunters are between 40-60 years of age with work effort declining after age 60 (Lee 1979). The decline in bone density of the Moita males after the age of 60-65

may indicate a similar reduction in hunting activity with advancing age. At the age of 60-65 males still had density values comparable to those of the 40-45 year old females. Bone density in males was less variable than in females and it decreased at a much later age than the drop in density shown by females after the age of 40-45 years. The sexual differences may result from the different physiological stresses experienced by females during the reproductive years, or the difference in hormones and the earlier decline in female hormonal levels than is shown by males.

As indicated in Figure 7, bone density in males inclined fairly steadily with age to the 60-65 age bracket in contrast to the non-linear decrease in the bone density of females. The low correlation between density and age ($r = 0.238$, $P = > 0.10$, $n = 17$) may have been influenced by the fall in bone density of the females between the ages of 20-30 and 45-60 years. The non-linear decrease in female density also may have prevented higher negative correlations between density and the Singh index ($r = - 0.488$, $P = <0.05$, $n = 19$). A tendency for density to increase with age is implied since osteoporotic bone loss is usually associated with advancing age (Singh et al. 1970; Singh et al. 1972). Lower grades on the Singh index have been associated with increasing age (Dequeker et al. 1976). Other studies have indicated a positive correlation between density and age in Black males; they showed no loss of density with increasing age (Baker and Angel 1965; Baker and Little 1965).

In general, Moita females had lower cortical bone density

when compared to males. The small number of females with age assessments (n = 5) as graphed in Figure 7, shows that between 20-25 years of age and between 40-45 years they have bone density exceeding that shown for males. A sharp decrease in the bone density of females in the 25-30 year range occurs when they were most likely to be in various stages of pregnancy or lactation. Although more calcium is stored in the bones during gestation than is required for fetal development (Irving 1973) it may have been inadequate to meet the stress of lactation, particularly if bone mineral was more available than calcium absorbed from dietary sources.

The drop in bone density in females during this five year period may be linked to child-bearing and to the extended periods of lactation reportedly practiced by many hunter-gatherer populations (Lee 1979; Coon 1976; Coale 1974). If calcium and phosphorus were being withdrawn from bone to meet mineral requirements during lactation it may not have been obtained entirely by endosteal resorption. The mean percentage of empty bone spaces was fairly evenly distributed throughout the cortex except in one individual with a higher percentage in the middle one-third of the cortex. In fact, in a comparison of the total cortical area the percentage of bone spaces was greater either in the middle one-third or in the outer one-third for all females including those without established ages. Not one female had a greater percentage in the endosteal area. It appears that if mineral was removed it could have come from within the total cortical area. This assumption is inconclusive as the counting

procedure necessarily included both resorbing and reforming cavities because of the bone staining problem. Supporting evidence, however, is provided by only a very slight decrease in cortical thickness. It may have been possible to verify the proportion of mineralized areas with microradiographs (Jowsey et al. 1965; Martin 1981) but facilities for this procedure were not available. Staining with Toluidine blue has been used in clinical settings to determine the ratio of mineralized to non-mineralized bone (Sabeau Pers. comm. 1985), but this method was not attempted due to the poor results obtained with the other stains.

Calcium and phosphorus can be retained in bone during lactation if the intake is above maternal requirements (Irving 1973). The phosphorus and calcium content in the shell-fish could have added a significant amount of mineral to the diet to meet lactational stress. The decrease in density suggests that adequate mineral may not have been available at all times for one or more reasons.

Seasonal occupation of the site might have prevented access to shell-fish in the off-season or the supply of shell-fish may have fluctuated. Personal preference and food taboos such as dietary restrictions associated with pregnancy and lactation (Coon 1976; Meehan 1982) could have lowered mineral intake. Vegetable sources of calcium and phosphorus may not have been as readily available depending on the season.

The decrease in the bone density of females aged 25-30 may have resulted from a delay in secondary mineralization associated

with an increased demand for calcium during lactation. During pregnancy minerals required to meet the needs of the developing fetal skeleton are met partly from dietary sources through intestinal calcium absorption and partly by mobilizing minerals from maternal bone (Parfitt 1981a). The process of calcium accumulation and an increase in bone mass begins early in pregnancy in preparation for fetal ossification in the third trimester and for later lactation. High levels of plasma vitamin D occur early but a high level of parathyroid hormone (PTH) normally occurs only in the later stages of pregnancy (Kumar 1980). This secondary hyperparathyroidism of pregnancy appears to be temporary, reversible and occurs in response to a lowering of blood calcium levels (Parfitt 1981a). As a result of the increase in new bone mass and increased bone turnover during lactation, secondarily stimulated hyperparathyroidism may have occurred in association with a delay in secondary mineralization. Hyperparathyroidism is associated with a delay in secondary mineralization (Wheater 1979; Parfitt et al. 1981b) and low bone density (Jowsey et al. 1965).

A similar reduction in bone density occurs in Moita males aged 30-35 years. Lower bone density at this age suggests that dietary calcium may have been inadequate. Access to a high calcium shell-fish diet may have been curtailed by seasonal occupation of the site or seasonal scarcity of these marine forms or perhaps males preferred to place a greater reliance on low calcium meat from terrestrial hunting. Insufficient vitamin D could also have decreased intestinal calcium absorption,

resulting in low plasma calcium levels (Guthrie 1975) and problems with mineralization (Parsons 1980). However, a deficiency would not be expected in the Moita hunter-gatherers who may have received an adequate supply of vitamin D through solar irradiation of the skin as a result of wearing minimal body covering especially in a mild climate. Calcium absorption by the intestine could have been impaired if grains had been included in the diet. Phytic acid, the organic phosphorus in grain, forms an insoluble salt with calcium (Irving 1973; Alvioli 1983). As a result the decrease in serum calcium levels could have initiated a secondary hyperparathyroidism reaction.

The physiological response of the body to a lowering of serum calcium levels leads to an increased secretion of parathyroid hormone (PTH) which stimulates osteoclastic resorption of bone. This reaction results in the release of calcium ions from the bone and raises serum calcium levels (Sinkiss 1975; Parsons 1980). Inadequate dietary calcium intake may have had an additive effect on females already under the stress of pregnancy and lactation. This could explain the sharper decrease in bone density experienced by the 25-30 year old females when compared to the decline in 30-35 year old males.

Disregarding ages and including the total sample, both males and females, there was a slightly higher number of resorption spaces in the outer periosteal one-third followed next by the mid-cortical areas. When all three cortical areas (PME) were compared there were fewer resorption spaces in the endosteal one-third indicating that calcium was withdrawn from the total

cortical area. Resorption is reported to be more diffuse and widely spread in hyperparathyroidism (Arnold 1970; Jowsey and Gordon 1971) but both formation and resorption are increased (Stout and Simmons 1979). When the number of resorption spaces in the endosteal one-third were counted separately in count three and the endosteal porosity was compared between each female and each male there was an increase in the number of spaces with age for both sexes. However, cortical thickness does not decrease. In fact, 30-35 year old males show an increase in thickness over the values of the previous 5 year period while females show only a very modest decline. This observation appears to support the suggestion that calcium was withdrawn from the total cortical area. If these results were caused by dietary deprivation it was short-lived, and temporary as bone density increases in females until age 40-45 and 60-65 years for males.

The subsequent steep rise in the bone density of Moita females after age 30 may have been in response to the heavier physical demands made on females during the reproductive cycle. The weight of the unborn child plus that of the older sibling carried was an addition to the normal weight of gathered food items or personal belongings carried during movement to new occupation sites (Lee 1979:314).

An increase in bone density in modern populations has been observed with an increase in physical activity (Nilsson and Westlin 1971) and with multiparity (Parfitt 1981a). A low level of mineralization has been noted during lactation but high levels were present in present-day females after child-bearing

(Goldsmith 1973). The burdens habitually carried by hunter-gatherer females, especially after the arrival of children, would have required strong bones to support the additional weight and to minimize the danger of bone fracture. An increase in bone strength evidenced by increased density and cortical thickness (Mazess 1983) may have compensated for the smaller bones of females.

A greater utilization of shell-fish with their high phosphorus to calcium ratio (P/Ca) may have occurred in females after child-bearing. Phosphorus is known to increase calcium absorption resulting in a positive calcium balance (Nordin et al. 1970; Irving 1973). It has been suggested that there is a good correlation between bone density and the calcium content of the diet (Williams et al. 1964) but other research has offered contradictory evidence (Smith et al. 1965; Exton-Smith et al. 1966).

The loss of bone density in females after 40-45 years of age and in males after age 60-65 may have been hormonally mediated. Diminishing levels of hormones begin earlier in females and mark the onset of menopause usually in the mid-40's. In males hormone levels are apparently maintained until age 60-65 but are then reduced to levels similar to those of premenopausal females. Although the age-related changes in the bone density of these Moita hunter-gatherers are similar to those reported for modern populations other factors could have made a contribution to the observed changes.

It is assumed that the end of reproductive life was also

accompanied by less strenuous work effort by females since physical activity increases the density of bone. Daily gathering would have continued but without the extra burden of child care. Presumably males over the age of 60 became less active in hunting resulting in less mechanical stress on bone. When bone is subjected to less stress and strain it becomes less dense (Albanese 1977). In addition, the intestine absorbs calcium less efficiently with increasing age (Bullamore et al. 1970; Gallagher et al. 1979). Ortner (1975) has also indicated that the rate of secondary mineralization decreases with increasing age. Since mineralization increases the density of bone (Parfitt 1983) any delay would result in bone that is less dense.

Asymmetry

The thin sections of cortical bone have provided evidence that porosity varies in different areas within individual thin sections. Porosity also differed between the right and left femora of one male and one female. These results tend to support Frost's (1964) observation that remodeling activity differs between different bones of the body and even within nearby regions of the same section. The number of transverse lines in the sections of right and left femora also differed. A comparison of right and left femora indicates that the most dense bone has greater cortical thickness. An increase in density and appositional growth has been observed in the dominant arm of tennis players (Stini 1985). Research on athletes has revealed that the denser femur was the leg of preference (Nilsson and Westlin 1971). They

also noted that there was increased bone density and bone mass with physical activity. Whether these results indicate the preferred side for weight carrying and therefore perhaps hand preference cannot be determined on the basis of two individuals and without comparison to arm bones. Perhaps the thicker denser left femur of the female indicates the side that supported child burdens. In the case of the male the right femur was thicker and denser possibly related to hunting activity.

Fluorescence

The accidental discovery of what appeared to be fluorescent labeling in the bone sections was an unexpected development. Fortunately, the only microscope available for use in this study was one that permitted settings for viewing with either ordinary light or fluorescent light, otherwise these results would not have been detected.

The origin of the apparent fluorescence in the osteons and the transverse lines in the cortex is unknown but several different possibilities might assist in explaining the fluorescence. Autofluorescence has been observed in the (c.45,000 year old) bone of Shanidar Man, a Neanderthaloid type (Blumberg and Kerley 1966), and in bone from the femoral shaft at necropsy (Prentice 1967). It was caused mainly by the collagen within the bone as decalcification did not change the autofluorescence. Older osteons are more highly mineralized and fluoresce more intensely than the less mineralized newly formed osteons (Prentice 1967). An increased rate of remodeling

such as is seen in the bone of young individuals or in hyperparathyroid states increases the number of new osteons that show little auto-fluorescence (Prentice 1967).

Tetracycline, an antibiotic that is used to label bone for studies on bone formation also produces fluorescence (Frost 1969). In contrast to autofluorescence, tetracycline does not fluoresce in highly mineralized older bone areas. Experimental study of other fluorescent markers, such as the red-fluorescing alizarin, has shown that the labels are incorporated into new forming bone (Lacroix 1971).

Alizarin binds to bone mineral through the mechanism of chelation (Myers 1968). The adjacent carbonyl hydroxy groups of the alizarin molecule can form a ring structure with metal ions such as aluminum and iron or with calcium, barium, magnesium, and others. Calcium forms a chelate ring by displacing the hydrogen which forms a bond between the oxygen atoms of the carbonyl and hydroxyl groups (Myers 1968).

Apparently a similar mechanism operates in the binding of tetracycline to calcium (Myers 1968). Tetracycline binds to calcium at sites on actively mineralizing osteons resulting in a fluorescent ring (Jowsey et al. 1970; Frost 1963). The ring appears yellow in fluorescent light and marks the leading edge of initial mineralization at the calcification front or demarcation line of a forming osteon (Lacroix 1971; Teitelbaum 1983; Frost 1963). Once the fluorescent ring is formed it becomes a permanent lifetime marker that can be detected in the same position in the osteon until the osteon is resorbed by osteoclasts (Parfitt

1983). The position of the labeled ring on the inner lamella or calcification front of an osteon depends on the stage of bone formation that the osteon is in when tetracycline is ingested. Tetracycline appears to have an affinity for collagen or the immature low density bone mineral at the demarcation line in osteons since labeling is less in bone mineral deposited several days previous to tetracycline ingestion (Parfitt 1983). The minimum width of fluorescence is usually about 4 um but if for any reason accumulation of mineral is unduly delayed, low density bone is increased and therefore a wider fluorescent ring may be found (Parfitt 1983).

There are two stages in bone formation: deposition of the collagenous (extra cellular) organic matrix by osteoblasts and then mineralization which increases the density of the new bone (Parfitt 1983). The initial mineralization begins in about ten to twenty days after matrix deposition and during the first four days or so reaches 70% of the maximum possible mineralization (Jowsey 1960; Frost 1963; Hall 1978; Parfitt 1983). It may take several months or years to achieve 90-95% of maximum mineralization (Jowsey et al. 1971; Parfitt 1983).

The fluorescent rings in the osteons of the Moita thin sections varied from 5-10 um in width suggesting that there may have been a delay in mineralization. An appositional rate of 1-1.5 um per day in osteon formation (Jaworski 1981) may indicate that periods of arrested growth lasted from 5-10 days. The SEM (U of A) also picked up differences in texture around the spaces in the bone. This result may indicate a greater or

lesser degree of mineralization or density around the spaces in the bone.

The results observed in the Moita thin sections depend on whether or not the fluorescence was caused by a tetracycline type antibiotic or by mold infestation. In the present research fluorescence labeling was observed under short wave blue light in the 380 to 420 nm wave length. Fluorescence produced by tetracycline can be observed at approximately 400 nm with short wave blue light (Bancroft and Stevens 1977). If the fluorescence on the inner lamellae of the Haversian canals was caused by an unknown tetracycline type antibiotic then it does indicate that new bone formation was occurring at the time of death. The growth arrest lines illustrated by the rings in the outer areas of several osteons were less fluorescent perhaps indicating that they may have been deposited prior to tetracycline ingestion since labeling is less in bone mineral deposited several days before ingestion of tetracycline (Parfitt 1983). However the labeled rings in the outer or deeper layers within the osteon might represent the fluorescence of previously ingested tetracycline while the labeled rings on the inner lamellae of osteons represents the most recent intake of tetracycline (Teitelbaum 1983). This suggests contact with the source of the fluorescence by in vivo ingestion rather than through post mortem soil contaminants such as bacterial or fungal sources.

Daily contact is not suggested as not all osteons show fluorescence. Several show double rings and two thin sections have osteons with three rings. This suggests periodic contact or

ingestion although it could indicate an osteogenic biorhythm, a cyclic pattern, because different bone forming sites may not be in the same phase of activity or inactivity (Tam et al. 1976). Frost (1969) states that labeling does not always occur because bone formation alternates with a rest period especially in older individuals and in some diseases.

The inner lamellae of Haversian canals in several osteons exhibited a wide diffuse ring. Diffuse fluorescence was also observed in several areas on the endosteal and periosteal borders of several thin sections. Fluorescent labeling of new bone on the periosteal surface has been observed after tracycline ingestion (Milch et al. 1958). It is possible that the diffuse areas in the endosteal and periosteal areas might have resulted from post mortem mold infestation as some areas of the thin sections appear poorly preserved. Some molds that cause bone destruction also produce a diffuse fluorescence on the bone surface (Hummert et al. 1982). For instance, fungi of the genera Mucor, Cladosporium, Candida, and Demtiacea resorb bone and some produce fluorescence but the fluorescence is diffuse and not patterned as in tetracycline labeled bone (Bassett et al. 1980).

Other fungal micro-organisms also have been implicated as causative agents in post-mortem bone decomposition and the production of a yellowish-green fluorescence. Piepenbrink (1986) isolated and cultured fungi recovered from exhumed skeletal remains. Inoculation of sterile bone samples with the fungus Stachybotrys cylindrospora produced several unidentified antibiotics which fluoresced a yellowish-green under fluorescent

light.

The location of the fluorescent labels on the inner lamellae of the Haversian canals and in the presumed growth arrest lines in the osteons suggests contact with the source of the fluorescence by in vivo ingestion rather than through soil contaminants such as bacterial or fungal sources. The regular outline or patterning, and position of the fluorescent rings is probably inconsistent with accidently caused fluorescence by microbes. The microfractures joining several osteons in one thin section (L1) exhibited no fluorescence but the inner lamellae of the osteons in this thin section were fluorescent. Fluorescence was also observed in transverse lines in the cortical bone of the thin sections.

The yellow-gold color of the concentric rings in the osteons and in the transverse lines appeared to be similar to the gold color observed in a teracycline sensitivity disc obtained from the Medical Microbiology department at the U of A (Jackson Pers. comm. 1986). Thin sections of five clavicles from the osteology collection in the Department of Anthropology at the University of Alberta, also showed yellow-gold fluorescence on the inner lamellae of the Haversian canals. It is quite likely that this sample of five clavicles from a modern population had received medical treatment with tetracycline at some point in their past. Although the fluorescence was brighter in the thin sections of clavicle than in the Moita bone this might be explained by the great age of the Moita material and perhaps by fading with time.

At the present time it is not known how the Moita population

could have come into contact with a possible antibiotic, especially one with fluorescent qualities such as tetracycline. The use of plants by herb-doctors in their herbal remedies occurred in Sumer and Egypt as early as 3000-2000 BC but apparently the practice originated from Asiatic sources where the remedies were used much earlier (Budge 1978). This knowledge soon spread to Nubia and to the Mediterranean areas. A practice in common use since "antiquity" in many areas of the world is the use of teeth cleaning sticks (Elvin-Lewis 1979). Pharmacological research has shown that many of the plants selected for this purpose have antibiotic properties. Weeds, grasses and common plants were used in the practice of folk medicine by early Europeans (Alland 1970; Isaac 1970). Many of the plants utilized in early Canadian native herbal medicines also have antibiotic properties (Chandler et al. 1979).

In recent years several authors have reported evidence of possible tetracycline labeling in human bone from prehistoric Sudanese Nubian archaeological sites (Bassett et al. 1980; Bassett 1981; Hummert et al. 1982; Martin et al. 1984). Evidently the practice of storing grain in mud bins provided a suitable culture medium for the growth of Streptomyces, a bacterium responsible for the production of tetracycline antibiotics. The Nubian diet is thought to have included beer and bread made from such stored grain.

Unlike the agricultural people of Sudanese Nubia, the Moita group were supposedly hunter-gatherers on the verge of transition to an agricultural subsistence economy. If they were in contact

with tetracycline, as the fluorescence appears to suggest, how did it occur?

The original and natural forms of the tetracycline family of compounds were produced by fermentation processes at a pH of 6-7 and a temperature between 25°C and 30°C, using Streptomyces species discovered during screening of soil samples for organisms with antibiotic properties (Mitscher 1978). A wide variety of substances have been used as sources of energy and nitrogen by the bacteria in the production of the tetracyclines. Energy has been obtained from starch, maltose, glucose, or mannitol, a slightly sweet crystalline alcohol found in many plants which, when oxidized, produces an aldose sugar called mannose. Nitrogen has been provided by meat extracts, molasses, amino acids, fish solubles, corn steep liquor or seed meal from which the oil has been removed (Mitscher 1978). Inorganic ions such as calcium carbonates are required to precipitate the tetracyclines as they are formed and to lessen their autotoxic effect on the Streptomyces.

Sources of nitrogen and carbon combined in cracked or ground cereal grains such as wheat, barley, oat, millet, or peanut meal have been utilized in the surface method of fermentation to produce feed-grain tetracycline antibiotics (Goodman 1985). In the surface method the ground grain is moistened and placed in flat trays along with the Streptomyces bacteria to produce tetracycline. Carbon from animal, vegetable, or peanut oils and carbohydrate from potatoes or yams have also been used in tetracycline production.

Preliminary data has provided no evidence of wild grain or vegetable remains of Moita (Roche 1972) perhaps because at poor preservation. Women may have gathered and stored wild grain, an assumption unverified by actual evidence, to extend their food reserves during seasonal scarcity. Such activity may have occurred at this site or elsewhere. Other groups with access to grain seeds may have provided an opportunity for trading or for an exchange of sea foods and grain. It is also possible that there were some initial attempts in grain growing at this time, perhaps as the first step in the process leading to food production. The storage of pine nuts, even for several days, might have provided the same results, however, this is uncertain. Pine nuts are suggested based on the probable identification of charcoal as (Pinus pinea) from the Muge sites (Roche 1972:86). of Amoreira and Arruda.

It is evident that energy, carbon and nitrogen sources are available from a wide selection of organic materials. Substances other than grain apparently could have provided these resources. Seeds and starch from vegetal sources must have been available at Moita along with meat, fish, and shell-fish. Inadvertent production of tetracycline could have been as simple as the methods of food preparation in practice at the time. Several foods mixed together and stored for a few days to produce a delicacy or to improve the flavors may have been implicated. Herbal potions prepared from plants and other materials by prehistoric healers for use in their medical treatments may have introduced the antibiotic, particularly if the mixture became

contaminated with earth since the tetracycline producing bacteria, Steptomyces, are usually found in the soil.

Although the level of tetracycline ingestion by an ancient population cannot be determined at present, there may have been a direct effect on infection rates. Because tetracycline is a broad spectrum antibiotic it is effective against many different pathogenic organisms (Mitscher 1978). Some investigators have observed tooth discoloration in young animals and humans given tetracycline (Ibsen et al. 1965; Wallman and Hilton 1962; Lofgren et al. 1968). Other researchers have detected areas of hypomineralization in the teeth following tetracycline ingestion (Nonomura et al. 1977; Lofgren et al. 1968). Neither of these conditions could be explored in the present study because tooth samples were not available.

CHAPTER V

HEALTH STATUS

Physical and Psychological Stress

The presence of stress in the population is supported by evidence from several sources. In a comparatively small subsample of the adult skeletal sample there are two females with evidence of parry fractures and one male who sustained a foot injury from an embedded stone bladelet (Lubell et al. 1986a). The fractures may have occurred during intragroup strife, but it also is possible that these injuries may have resulted from occasional intergroup aggression. There may have been competition for subsistence resources or occupation sites.

Any psychological or physical stress such as fear, injury, pain, or infection results in a release of cortisol, the major glucocorticoid in the human body (Selye 1956; Guyton 1976). The frequency of aggressive-defensive encounters in the population can never be determined with certainty, but even the fear of periodic forays may have been sufficient to maintain psychological stress levels. If this did occur the result may have been higher than normal levels of cortisol secretion in at least some members of the population.

Males over 40 may have been particularly stressed as evidenced by the loss of cortical bone thickness. The significance of excess cortisol with respect to the present study lies in its effects on bone biology. Osteoblastic activity is decreased along with an increase in osteoclast activity (Hahn et

al. 1974; Guyton 1976). The long term effects of chronic cortisol hypersecretion may be the development of osteoporotic bone loss (Manolagos et al. 1979; Deluca 1982). Excess cortisol in the blood arising from both psychological and nutritional stress has also been implicated in a high level of dental caries (Wing and Brown 1979). The high level of dental caries in the permanent teeth (13% of 648) of the adults at Moita (Lubell et al. 1986a) may have been influenced by circulating serum cortisol levels, however, other factors could be involved. Perhaps the high carbohydrate content of mussels (Buikstra 1984) was one component involved in the development of dental caries. (Mytilus edulis) has 3.920 grams of carbohydrate per 100 grams of edible portion compared to deer (Cervus Elaphus) meat which has none (Souci et al. 1981). However, the contribution of mussels to carbohydrate intake would be low in relation to fruits and vegetables (Souci et al. 1981) and would depend on the amount of mussels they ate in comparison to other foods.

Physiological Stress

Physiological stress (Buikstra and Cook 1980) or nutritional stress (Huss-Ashmore et al. 1982) is indicated by linear enamel hypoplasia and Harris lines. Linear enamel hypoplasia is present in a frequency of nearly 50% (248/515) in the adult dentition of both males and females of a larger sample drawn from the population (Lubell et al. 1986a). There is evidence of Harris lines in several femora but their frequency and analysis awaits further study. Enamel hypoplasia (Cook 1979; Wing and Brown

1979; Cassidy 1980) and Harris lines (McHenry 1968; Gindhart 1969; Garn 1969; Kühl 1980; Hunt and Hatch 1981; Maat 1984) have been attributed to periods of illness, emotional stress, or food scarcity during childhood and perhaps adolescence. Longitudinal growth of long bones is temporarily arrested until these intervals are ended. The activity of cartilage cells at the epiphyseal plate is halted but the activity of osteoblasts at the juncture of the cartilage plate and the metaphysis continues, resulting in the formation of a thin layer of bone called the primary stratum (Steinbock 1976). After the intervals of illness or food deprivation, the osteoblasts immediately begin to build bone on the primary stratum resulting in a thick secondary layer that is seen as a transverse line (Steinbock 1976). The formation of these lines becomes evident only in the post-recovery stage when growth recommences with a return of appetite or when food resources become available. In many instances Harris lines do not form after illness and sometimes they form when there has been no illness (Marshall 1968; Gindhart 1969).

Harris lines can be observed in some of the Moita femoral x-rays although only one femur appears to have a line extending across the marrow cavity near midshaft. Transverse lines in the thin section of cortical bone in this particular femur do not match the number of Harris lines in the x-ray. The probability of finding Harris lines in cortical bone at midshaft is no doubt low since most researchers report their presence in cancellous bone at the distal ends of long bones (McHenry 1968; Steinbock 1976). One of the long bones in McHenry's (1968) photographed x-rays

does appear to have one line near midshaft although it is not mentioned. A small 9 mm circular bone core probably has even less chance of sampling an area at midshaft that contains Harris lines particularly since the core consists of cortical bone.

Post-mortem bacterial or fungal invasion has to be considered in studies of archaeological bone. The easiest route for microorganisms to gain access into cortical bone would be through the Haversian canals or fracture lines. There is, however, no evidence in the clear areas of outward destruction of Haversian canals other than the normal resorption tunnels occurring in bone. The cortical transverse lines give no indication of being former fracture lines with later calcification by minerals from the surrounding soil and shell material in the midden. In fact, the lines appear to follow the normal anatomical deposition of the matrix and are still observable under polarized light. This suggests that these lines are not artifacts and that they are part of the bone microstructure. They are too straight and are parallel to all the other lines in the section to be fracture lines. It would be unusual for bacteria to form the pattern observed in the bone even by chance.

There are several research-related reports in the literature that refer to lines in the matrix of compact bone. These are highly calcified thin lines found in cross-sections from the midshaft of femora and tibia of young calves and humans apparently free of bone disease. The lines, called "Mittellinien", are layers of primary bone regularly dispersed in

the outer middle and inner zones of compact bone (Pugliarello et al. 1973; Ascenzi 1983). They are present in fragments of interstitial periosteal lamellar bone left between secondary osteons.

"Mittellinie" are short lines and therefore not completely satisfactory as an explanation of the long transverse lines in the thin sections although they might perhaps explain some of the short lines. The transverse lines are not confined to the middle zones or to the small areas between secondary osteons. In some instances, the lines cross the entire width of the thin section and may be found anywhere from the periosteal surface to the cortical endosteal border. The association of "Mittellinien" with the healthy young and their presence between secondary osteons suggests that their short length may be the result of remodeling activity during the growth process. Unlike "Mittelinien" the long and short transverse lines in the cortical bone from Moita were present in 12 individuals of all ages from 15-20 to 40-45 years of age. The persistence of these lines into adulthood suggests that the lines are probably not Mittellinien but they may indicate physiological stress and a problem with bone remodeling.

The observation of similar long transverse lines in thin sections prepared from cross-sections of adult tibial diaphysis from a present-day human has been discussed in another study (Lacroix 1971). They were called "arrest lines" or "resting lines", and growth arrest lines in osteons were originally compared to these lines. Lacroix (1971) theorized that at some point in the past osteoblasts temporarily stopped depositing

bone; the lines became hypercalcified during the halt and at a later date the osteoblasts resumed their normal function of depositing bone. During this interval no resorption of bone occurred (Lacroix 1971).

A broad expanse of bone outlined along both sides by such transverse lines was observed in one thin section from a female in the Moita sample. The distance between the two lines measured 1500 um. There were no resorption cavities, osteons or forming osteons within their boundaries. Evidence of previous osteoblastic activity was provided by the presence of osteocyte lacunae in the lines and in the area bounded by the lines. Thin sections from two others, both males, had similar areas but the distance separating the lines was 800 and 900 um.

For these individuals it appears that there was a lengthy period with no arrested growth. The normal bone formation rate of lamellar bone is approximately 1-2 um per day (Lacroix 1971; Sissons 1971; Bloom and Fawcett 1975; Jaworski 1981). Assuming an average growth rate of 1.5 um per day the interval separating the two lines of the first individual was approximately 2-2.5 years and 1-1.5 years for the latter two individuals. These intervals may indicate that these persons enjoyed relatively good health during this time but other closely spaced lines formed later suggests that episodes of stress occurred much more frequently during the following years.

The transverse lines measured a consistent 10 um in thickness indicating that arrest periods may have lasted approximately 6-10 days with subsequent recovery until arrested

growth reoccurred. The two right femora had several lines measuring 5 um but the remainder were 10 um thick. There was considerable variability in the distance between the lines but many were separated by short distances suggesting that the 12 individuals with the transverse lines may have been under frequent nutritional stress. A few lines were so close together that they appeared as double or triple lines. The distances between most of the lines were too irregular to conclude that dietary deficiencies or disease occurred on a regular basis.

It is suspected that the transverse lines observed in the thin sections may be attributable to arrested growth during periosteal bone apposition. There is a slight indication of a decrease in the number of lines with increasing age ($n = 5$, $r = -0.435$, $P = > 0.10$) although the number of individuals with both lines and age assessments is small. A comparison with the Singh Index and lines ($n = 9$, $r = 0.319$, $P = > 0.10$) also indicates that the number of lines tend to decrease with age possibly because of resorption. However, neither of these correlations was significant. Some lines are interrupted by resorption cavities and osteons but endosteal resorption would be more likely to remove the lines during marrow cavity widening. Since periosteal bone apposition continues throughout life, although at a slower rate (Frost 1973), these lines would continue forming during each episode of growth arrest but they might be less frequent with aging. The number of lines and stature were highly negatively correlated ($n = 7$, $r = -0.804$, $P = < 0.05$). Taller individuals were associated with fewer lines while shorter individuals tended

to have more lines. These results suggest that growth in femoral length was periodically slowed or halted while growth in cortical width, as evidenced by cortical thickness, continued. The continued formation of these lines in adulthood would be unrelated to long bone growth, if these lines do represent periosteal apposition, but lines near the endosteal regions may represent the lines formed earlier as a result of problems that occurred during growth particularly during the adolescent growth spurt.

The cortical width of these 12 individuals was not associated with stature and showed only a low correlation with the number of lines ($n = 11$, $r = 0.383$, $P = > 0.10$). One young male, (L57) the youngest in the sample at age 15-20, had 9 of these transverse lines in a cortex measuring slightly below the mean shown by males. This may indicate that he experienced problems during growth and perhaps was still having problems when he died. Density and the number of transverse lines in the cortex showed no association probably because the complete range of density values from the lowest to the highest were included in this group.

The transverse lines suggest that the experiences of this segment of the population were different from those of the main group. Since both males and females of different ages, but mainly under 40, were included in this group it appears that the stressor was not based solely upon sex or age. The evidence of growth arrest based on the cortical lines suggests that the problem may have had a nutritional basis or was possibly related

to illness. Why this group had these lines, while the main group lacked these lines, is unknown. They may have been an independent group unassociated with the main group but burial into the same midden probably negates that possibility.

Status-related nutritional stress might account for these lines since none appear in the bone of individuals in the larger group. In times of scarce resources others in the main group may have had priority to subsistence items. Despite the presence of growth arrest lines in the cortex, rapid periosteal apposition must have occurred during each phase of recovery as these individuals show a slight gain in mean cortical thickness (6.218 mm) in comparison to the mean (6.201mm) for the entire group. Nordin's index also reflects this small gain with a correlation of 0.450 and a mean of 63.2 compared to 62.6 for the total group. The individuals with lines averaged 7 cm taller (163.7 cm, sd = 4.19) than those of the main group (156.17 cm, sd = 7.75) perhaps reflecting greater nutritional requirements and increased sensitivity to nutritional stress.

Student's t was used to test whether stature was significantly different between the group with lines and those of the larger group without lines. The results suggest that there was a significant difference in mean stature ($t = 2.367$, df. = 17, $P = 0.05$). The F test was used to compare stature means. A large F value was obtained, (Table 7, page 153) implying that there was a significant difference in mean stature between the two groups ($F = 11.83$, df. = 3, 15, $P = 0.05$). In fact this value for F was also significant at both the 0.01 and 0.005 levels.

Both tests suggest that mean stature differs between the group with lines and the group without lines. The large variation in stature is difficult to explain. The differences might be the result of genetic factors, diet, or in-migration of the group with lines during some previous period.

The possession of thick cortical bone by the people of Moita (Lubell et al. 1986a) may be genetic in origin or it may provide an indication of the general health and nutritional status of the population (Martin et al. 1981). Thin cortical bone has been observed in severe physiological stress (Martin and Armelagos 1979; Huss-Ashmore 1981). Skeletal tissue responds to nutritional stress (Huss-Ashmore et al. 1982) with marked effects on cortical thickness (Hatch et al. 1983). Thick cortices have been associated with osteosclerosis (Stout and Simmons 1979). Increased cortical width, density, and thickening of the periosteum has also been reported in fluoride-induced osteosclerosis (Guthrie 1975; Albanese 1977). However, in view of the apparently high level of dental caries in the population (Lubell et al. 1986a) fluorine probably was not involved. Cook (1984) cites Garn and Solomon (1981) in reporting that obese individuals have thick cortical bone but Cook adds that this occurrence would be more likely in sedentary groups.

Thick cortical bone has also been noted as a consequence of vitamin A deficiency (Wolbach 1947; Navia and Harris 1980) which continues to be a major health problem in developing countries (Oomen 1976; Srikantia 1982) and has also been observed in western Europe (McLaren 1967). As late as 1969, 10% of Canadians

had no liver stores of vitamin A and 20% had values equal to those at birth (Campbell 1973; Guthrie 1975). In the United States, 33% of the population was affected, particularly those with reduced food intakes such as low income groups or those on reducing diets (Guthrie 1975; Wilson et al.1979; Owen and Owen 1982). Hunters and gatherers also may have been at risk during periods of food scarcity because of seasonal variation in the food supply.

Preliminary assessment of the available archaeological data on subsistence resources indicates that a periodic deficiency of vitamin A could have existed. Mussels and snails have no vitamin A activity and clams and oysters contain only small amounts (Guthrie 1975; Adams and Richardson 1980; Pennington and Church 1980). One pound of clams and 13-19 medium sized oysters (1 cup) provide approximately 480 and 740 international units (IU) respectively, values far below the recommended dietary allowance of 5000 IU per day for males and 4000 IU for females (Guthrie 1975; Adams and Richardson 1980). Recommended allowances in Canada and the United States, however, may differ from standards recommended for Europeans. Seasonal availability and preference may have been a factor in the utilization of clams and oysters. Crabs contain 2170 IU per 3.5 ounces (Pennington and Church 1980) and might have provided significant amounts depending on their availability and the quantity consumed. The vitamin A content in crabs may be variable as Guthrie (1975) and Adams and Richardson (1980) indicate little or no vitamin A content.

There are two main sources of vitamin A (Ganguly and Murthy

1967; Roels 1967; Guthrie 1975). The biologically active form is found preformed only in foods of animal origin such as eggs, heart, kidney, liver, and milk. Since the liver stores vitamin A, it is the richest source but the content varies with the diet and the season, with a lower content stored in winter. The amount in the liver rises with the increasing age of the animal and varies with the species (Wilson et al. 1979). Fish liver oil from many fish is rich in vitamin A, however, the liver of freshwater fish has only 33-40% the potency of vitamin A (Ganguly and Murthy 1967; Guthrie 1975). The precursors of vitamin A, the carotenes, are found in the fruits, leaves and roots of many plants. Their potential value depends on the ability of humans and animals to convert the carotene to vitamin A in the intestinal wall where vitamin A is absorbed. High intake levels are necessary in order to meet daily requirements as only approximately 25-50% of dietary carotene is converted and carotene has approximately 50% the potency of vitamin A (Wilson et al.1979).

Problems arise when more than 50% of vitamin A is derived as carotene from vegetable material (Srikantia 1982). The high level of vegetable plants in the diet of many hunter-gatherers (Jelliffe et al. 1962; Maddock 1972; Lee 1979) may have placed many at risk of developing periodic deficiencies especially since most plants are seasonally available. The Hadza of northern Tanzania, with a diet composed of a high level of vegetable foods, showed evidence of vitamin A deficiency in both younger (13%) and older (17.3%) adolescents (Jelliffe 1962). The introduction of weanling diets based on vegetable foods (Cassidy

1980) may have increased the risk of developing a deficiency in many hunter-gatherer groups, however, prolonged lactation does offer some protection (Srikantia 1982).

There is at present no data available on the type of plants that may have formed a substantial part of the diet at Moita. Since vitamin A is fat soluble, the low fat content of vegetable protein (Srikantia 1982) may have lowered the absorption of the vitamin. In addition to the problem with absorption when dietary fat content is low, the rapid passage of high fiber plant foods through the intestinal tract tend to reduce the amount of carotene released from ingested food. Cooking and drying during food preparation also tend to decrease the value of carotene in plant foods by at least 15-35% (Wilson et al. 1979). It is possible that the hunter-gatherers of Moita, like the North West Coast Indians and the Yurok of California, considered plant foods as relatively unimportant (Oswalt 1978). "Nowhere did vegetable foods loom as a very important part of the diet,...." (Newcomb 1974:214) for the peoples of the North West Coast.

The low protein and fat content of mussels (Parmalee and Klippel 1974) may have restricted the formation of a protein-complex (Guthrie 1975) that is required for the release of vitamin A from liver stores and transportation to other areas of the body. A combination of low fat mussels and plants may have seriously decreased the biological availability of vitamin A from carotenes. Liver from terrestrial animals would have provided the major source of vitamin A with the addition of lesser quantities from kidney and heart (Pennington and Church 1980).

Vitamin A deficiency has been associated with linear enamel hypoplasia (Sweeney et al. 1969; Albanese 1977; DeLuca 1980; Blakey 1981; Whitney and Nunnelly 1981) and Harris lines (Wolbach 1947; Goodman and Clark 1981). Vitamins C, and D deficiencies in childhood have also been associated with linear enamel hypoplasia and Harris line formation (DeLuca 1980; Blakey 1981; Goodman and Clark 1981).

Although vitamin deficiencies do not usually occur in isolation (Guthrie 1975; Steinbock 1976), there was no apparent vitamin D deficiency indicated by rickets or bone deformation (Stini 1973). This may be because adequate exposure to ultraviolet radiation provided the major source of vitamin D (Albanese 1977; DeLuca 1982). It seems unlikely that vitamin D deficiency was a problem for the hunter-gatherers of Moita: the outdoor life-style and perhaps minimal body covering probably ensured sufficient exposure to ultraviolet radiation to produce vitamin D in the skin. This is the major source of vitamin D and usually eliminates the need for further dietary additions (Albanese 1977; DeLuca 1982). The thin cortices in long bones and subperiosteal hemorrhages observed in vitamin C deficiency (Vilter 1967; Steinbock 1976; Albanese 1977) were not obvious in the adults of this sample. The small amounts of vitamin C contained in the meat of liver and clams (Guthrie 1975; Adams and Richardson 1980) may have protected them against a deficiency when plant sources of vitamin C were in short supply.

Nutritional stress may be related to the transverse lines in the cortex and may have involved a vitamin A deficiency. The

variable amounts of vitamin A in the liver of different species may have provided insufficient vitamin for all individuals. The success and the frequency of hunting may have had an influence on the quantity of vitamin A available to the group. The 12 individuals with transverse lines may not have been accepted by the main group since they include the two females with parry fractures and the male who was shot in the foot. They may not have obtained a share in the vitamin containing organs after every hunt. Hunters have been known to eat the liver at the kill-site (Lee 1979) and bring the leftovers back to camp (Jelliffe 1962). Depending on the amount consumed and stored in the liver the successful hunters may have obtained protection against a deficiency. The total group size numbered approximately 57 (Lubell et al. 1986a) and even 45 of these individuals would not have obtained sufficient daily requirements of vitamin A from 16 ounces of liver from the animals they hunted even if the values were comparable to beef or lamb liver. The vitamin A in the liver of Aurochs is unknown but may have been comparable to that of modern beef animals. Since 33.4% of the faunal bones recovered from the site are those of rabbits, the small-sized liver may have provided insufficient vitamin A for everyone and it may not have been eaten by the people.

Since growth arrest lines in osteons have been compared to the growth arrest lines in cortical bone (Lacroix 1971) and to Harris lines of arrested growth (Stout and Simmons 1979) it seems reasonable to assume that similar factors influenced the formation of cortical growth arrest lines. It appears that these

12 individuals endured illness or nutritional stress severe enough to cause episodes of growth arrest and death by age 40-45. A reduction in food intake during illness or when seasonally produced food resources are in limited supply increases the risk of a deficiency.

During intervals of vitamin A deficiency long bone growth may be slowed or halted because of an imbalance between resorption and formation (Guthrie 1975; Bloom and Fawcett 1975; Guyton 1976; Whitney and Nunnelly 1981). Resorption by osteoclasts is reduced but osteoblasts continue to build bone on periosteal surfaces (Vaughan 1981). Based on animal studies of skeletal growth in vitamin A deficiency states, long bones are short and thick due to the failure of endochondral bone growth and continued appositional bone growth (Wolbach 1947). The continuation of long bone growth in preference to cortical width under conditions of nutritional stress (Huss-Ashmore 1981) is unlike that observed in vitamin A deficiency where there is a retardation of long bone growth but appositional growth continues. The periosteal overgrowth observed in vitamin A deficiency (Irving 1973) may have contributed to the thick cortical bone at Moita perhaps even in those less obviously affected. In animal studies, regardless of age, periosteal overgrowth is the major effect (Barnicot and Datta 1972).

The possibility of a vitamin A deficiency could have had several potentially serious consequences for the people at Moita. The ability of females to conceive is affected by seasonal food shortages (McElroy and Townsend 1979) possibly as a result of a

reduction in vitamin A intake. Animal studies have shown that a vitamin A deficiency is associated with a reduction in the fertility of both males and females along with lower reproductive success (Moore 1967; Guthrie 1975; Guyton 1976; Navia and Harris 1980). Seasonal periods of food scarcity could have had direct effects on the birth rate and population size. It is tempting to suggest that long lactation may not have been the only influence on birth spacing.

Vitamin A deficiency produces other serious health problems. There is an increased risk of infection in vitamin A deficient individuals. Respiratory and gastrointestinal tract infections (Guthrie 1975) may have had serious consequences on health and longevity particularly for infants and youngsters. The mortality rate is unknown but these infections may have been the leading cause of death in all age groups. Based on examination and study of lung tissue from a series of mummies from Peru and Chili, Allison (1984) reports that the major cause of death was pneumonia. It was also suggested that infection and pneumonia have probably been the leading cause of death for the past 8000 years.

The generally low incidence of pathology in the skeletal material at Moita (Lubell and Jackes 1985) may have been due to the apparent ingestion of tetracycline evidenced by labeling in the bone. Ingestion of tetracycline may have reduced the seriousness of infections and shortened recovery time. The development of bacterial resistance with low dosages (Mitscher 1978) is a possibility that might have reduced the effectiveness

of the antibiotic. Antibiotic resistant bacteria have been reported in communities with no prior exposure to commercially produced antibiotics perhaps as a result of bacterial exposure to naturally occurring streptomyces produced antibiotics (Davis and Anandan 1970). Inadvertent production and ingestion of antibiotics such as tetracycline by the people of Nubia and Moita and perhaps by other groups may explain the discovery of antibiotic resistant bacteria in later generations. The dose-related levels ingested at Moita are unknown but the general health of the population may have improved along with longevity.

Osteoporosis

The prevalence of osteoporosis as indicated by the Singh Index (Singh et al. 1970; Singh et al. 1972) was low. Singh grades 2 and 3 osteoporosis were present in 1 male and 2 females. Borderline grade 4 osteoporosis included 1 female and 3 males. The low incidence of osteoporosis (9%) implies a reduced risk of femoral neck fractures and disability for the majority of the population. The value of the index lies in its ability to identify individuals potentially at risk of sustaining a femoral neck fracture (Wahner et al. 1973; Dequeker et al. 1976). According to Nordin's Index (Barnett and Nordin 1960) determined from antero-posterior radiographs of the femur (Lubell et al. 1986a), osteoporosis was not a problem in the population. There were no individuals with values of less than 46, the value considered to be the point separating osteoporotics and normals.

The lack of agreement between the Singh Index and the Nordin

Index may be due to the different rates of bone loss between cortical and trabecular bone. Trabecular loss occurs at a faster rate than is observed in cortical bone (Mazess 1983). Other studies have indicated that changes in trabecular bone show no relationship to changes observed in the cortical bone of the same sample (Mielke et al. 1972). There was also a low correlation between the Nordin Index and directly measured cortical thickness ($r = 0.380$, $P = < 0.05$, $n = 28$) and no correlation between the index and endosteal porosity. Apparently cortical thicknesses measured on radiographs are not accurate representations of the actual directly measured thickness of cortical bone (Van Gerven et al. 1969) because of the inability of radiographs to distinguish early endosteal bone loss from compact bone (Mazess 1980). However, a combination of radiographs and direct measurement of thickness can indicate the amount of bone loss due to endosteal remodeling (Mazess 1980).

The severity of osteoporosis can also be determined by measuring cortical thickness and porosity (Martin 1981). Direct measurements of cortical thickness on the bone cores and calculation of porosity in the thin sections indicated that females were unaffected by osteoporosis. Males showed osteoporotic bone loss by age 45-50 but there was no accompanying loss in density. Since there is a loss of density associated with age-related osteoporosis (Albanese 1977) these results suggest that factors other than increasing age may have been involved. A change in nutritional status might account for the early bone loss in males.

According to Thompson et al. (1981) cortical bone density outside of certain limits may indicate pathology or alterations due to the effect of interment. Calculated values for bone density in many populations of different ages and sex has indicated that the average bone density is 1.85 gm/cm^3 with a range between 1.60 gm/cm^3 and 2.10 gm/cm^3 (Thompson et al. 1981). Values outside the lower or upper limits are either pathological or modified because of mineral replacement or removal by ground water.

The bone density of the Moita femora at 2.12 gm/cm^3 for males (n = 19) and 2.09 gm/cm^3 for females (n = 11) is at the outer limits of normal density just described above. In this case the mean obscures those values falling outside the range. Three males including the youngest had density values below 1.60 gm/cm^3. Seven males and five females had values above 2.10 gm/cm^3. The thick cortical bone may account for these results or bone minerals may have been replaced or added to the bone.

SEM analysis revealed a high calcium content with some phosphorus and very small amounts of other minerals such as iron and copper. The possible effect of interment on bone density could have been tested by correlating the length of burial time and density but a lack of records prevented this type of analysis. Since burial was into the shell midden calcium may have leached out of the shells and into the bone. Carbonates are known to replace phosphates in bone (Race et al. 1972). Antemortem pathology cannot be discounted in view of the high density and generally thick bone of the Moita people. Turnover rates could

not be measured but as previously discussed high turnover rates are associated with less dense bone and low rates are related to high density values.

CHAPTER VI

SUMMARY AND CONCLUSION

Age estimates could not be determined by counting osteons in the thin sections prepared from the femoral bone of the Moita do Sebastiao population. Extensive areas in the thin sections were affected by an ash-gray discoloration. As a result of this stain the osteons were obscured and osteon counts could not be obtained. If the cement lines of the osteons could have been outlined with haematoxylin and eosin a count may have been possible but attempts at staining were unsuccessful.

Efforts to remove or lighten the stain were unsuccessful despite the use of several different methods. It is assumed that a combination of marine shell, soil mineral and organic material in the midden matrix produced unknown chemical reactions that resulted in staining of the skeletal remains. Whether all osseous material in shell middens is affected in this manner is unknown but the skeletal remains associated with shell material in three different sites were affected by similar discoloration. The discoloration was a major problem in this research and needs to be resolved as it presented serious limitations for microstructural assessment.

Although the microstructural features in some areas of the thin sections were disrupted, distorted, or swollen they were fairly well preserved in other areas. Ultrasonic cleaning in distilled water might have been responsible for the distortion but dehydration in alcohol and xylene should have minimized these

effects. The bone tissues might also be showing changes resulting from diagenic processes.

Differential areas of bone hardness in the thin sections resulted in poor section quality and breakage during thin section preparation. Laboratory stains were unable to illuminate the cause of the ash-gray stain or the reason for the variation in bone tissue hardness. Perls' Prussian Blue identified iron as a thin rim on the periosteal surfaces, but since iron was not present in the remainder of the thin sections, it appears that iron made no contribution to the variable areas of hardness within the thin sections. The high calcium content of the bone as indicated by von Kossa's stain and the SEM may have produced the uneven areas of hardness, although this is not certain.

There is no possibility of obtaining quality thin sections from fragile archaeological material such as the Moita skeletal remains or the Algerian human ribs when this material is subjected to grinding and polishing. It is too destructive to unprotected bone tissue, is extremely time consuming, and a great deal of valuable tissue is lost. The sawing procedure used with the bone cores was fairly nondestructive; the width of the cutting edge of the circular saw blade accounted for the only tissue loss.

The most practical solution is to embed fragile skeletal tissue. Skeletal material is then protected, and there is less tissue wasted and time lost. A 1 mm thick section of bone removed from the 9 mm bone cores and embedded in Ward's Bio-Plastic provided several thin sections 80-100 um thick. Embedded

bone material is preserved as a permanent record of the skeletal sample. Both the remaining unembedded bone core samples and the embedded samples can be restudied with newer techniques as they become available or they can be reanalyzed by other researchers in the future.

The removal of bone core samples is less destructive to valuable skeletal remains than the removal of cross-sections but there may be a loss of information in comparison to complete cross-sections. Removal of core samples from medial, lateral, posterior and anterior sites might overcome some of the problems and still retain the normal bone length, thickness and other information for future analysis. The cores should be marked on medial or lateral sides along with a mark on the superior side to maintain correct orientation to the intact bone and to provide as much information as possible. Since the removal of small bone cores is less invasive and destructive there is a greater possibility that permission for their removal can be obtained. This appears to be of particular concern when the skeletal tissue involves a foreign country or valuable museum collections.

Although a bone core is a small sample of the human skeleton it can provide information on the health status of a population. The information obtained from histomorphometry can be combined with data from other techniques such as radiographs, scanning electron microscopy (SEM), trace element analysis and other methods to provide a more complete picture of health.

For instance, SEM with its x-ray equipment provided information on the trace elements in the femoral and rib samples.

Although the ratio between calcium and phosphorus was not determined, SEM did indicate a higher level of calcium than phosphorus and noted that other elements were present only to a limited extent. The evidence of bacteria associated with the bone might not have been obtained if SEM had not been used. SEM can be a valuable supplement to other techniques when gathering information from archaeological bone.

Since histological analysis for purposes of age determination may not be possible with all archaeological material other techniques must be utilized or new methods developed. Endosteal porosity and cortical thickness are not dependable age indicators as nutritional problems or metabolic diseases can alter their values. Although the youngest definitely aged individual had a low score for endosteal bone spaces this might not be a consistent finding in other individuals.

It was emphatically realized how important the ages of individual skeletal remains are to any comparison. The lack of estimated ages was a severe limitation when a comparison of results was attempted. It was for this reason that Jackes' (Lubell et al. 1984) data on estimated ages was tested in this research.

Although the age estimates based on attrition grades are still in the preliminary stage of assessment they do appear to be reasonably accurate. Data presented in the literature on cortical bone changes occurring in modern populations and the average age of onset of these changes tends to support, in many instances, the age changes observed in the Moita bone. Some of the

differences in age changes between modern populations and the Moita population may derive from the age estimates themselves or the microscopic technique of measurement. It is also possible that any lack of agreement may be the result of genetics, diet, or population differences in the activity levels of hunter-gatherers.

The menopausal loss of bone density by Moita females after age 45 and males after 65 is in close agreement with modern data. Maximum cortical thickness is reached by age 40 in Moita males and in present day males and females. Moita females show no loss in cortical thickness nor increased porosity but may not survive long enough for these changes to become evident. Moita males show early loss in cortical width along with an increase in porosity perhaps as a consequence of genetically determined early aging. Moita females show the changes in porosity and density that might be expected during the reproductive and lactation cycles. In addition, sexual differences are clearly apparent.

It may be unrealistic to assume that biological processes in prehistoric populations of 7000 BP were identical to those of modern day populations. The fact that females in this research did not lose cortical thickness while males experienced early bone loss in comparison to present day research findings may reflect a difference in the biological response to stress.

Assuming that biological responses to stress were similar and that diet was important in maintaining cortical thickness it then becomes apparent that males were undergoing some type of change. Rather than cortical bone loss from the effects of early

aging, males may have been experimenting with, or adapting to, newer dietary additions. The stability in female cortical thickness in comparison to males suggests that females were generally following the usual dietary practices. If deviations did occur there was no resulting bone loss.

There may be an additional explanation for the absence of cortical thinning and the increase in cortical thickness in females. An accumulation of endosteal bone during pregnancy may not have always been lost with subsequent lactation because of loss either before birth or in early infancy. Endosteal loss due to osteoporotic changes with increasing age may not have occurred because females did not survive long enough or the menopause may have occurred somewhat later than it does in present day populations. The physically active lifestyle of a hunter-gatherer female may have had an effect on the thickness of the cortices.

The actual nutrient intake is unknown but there is an indication of a possible dietary vitamin A deficiency. An increase in the number of infections, lowered fertility or premature termination of pregnancy may have had quite an impact on the health and size of the population.

The evidence suggests that tetracyclines were ingested. If this did occur, the frequency of infections might have been decreased, although the effect of antibiotics can be lessened with the development of bacterial resistance. If samples of adult teeth had been available for thin sectioning they could have been examined for evidence of tetracycline banding. The presence of banding would have suggested contact with tetracycline during

the formative stages of permanent tooth development, and it might have been possible to calculate when that contact first occurred.

The source of the apparent tetracycline labeling in the bone is a problem. Whether grain, herbal remedies or some other substance provided a suitable culture medium for the production of the apparent tetracycline has not been determined. The consistent pattern of fluorescence in the arrest lines of the osteons, the inner lamellae of Haversian canals and the cortical transverse lines are all suggestive of in vivo ingestion. This type of pattern appears to be similar to the labeling found with tetracycline ingestion in modern medical treatment. Even though some lines varied in width most were sharp fine lines with no diffusion of their margins.

If the fluorescence was due to autofluoresence there would have been a more diffuse coverage of the bone. Several areas of diffuse fluorescence on the periosteal and endosteal borders might be attributed to autofluorescence, but also to bacterial or fungal sources. The bacteria associated with one bone sample might have produced this type of dispersed fluorescence but they probably were not responsible for the fluorescence of the arrest lines because of the consistent and regular patterning of the lines.

The bacteria may have had an association with disease and illness in the population but this could not be determined. Although they were tentatively identified as bacteria, not one of these could be isolated from the bone scrapings in order to obtain a positive identification. Any consideration as possible

disease agents would be merely speculative, especially as they could have become associated with the bones during any post-depositional stage.

The transition from hunting and gathering to food production appears to have been an unsettled and stressful period for the population at Moita. Illness or nutritional stress during the growth period is reflected by linear enamel hypoplasia and Harris line formation. The presence of these markers in both the bones and teeth of adults indicates that these individuals survived into adulthood despite stress-related events. Stress was not confined to the formative years since adults show evidence of physical injury and growth disruption as indicated by the cortical transverse lines and osteonal growth arrest lines in the femora.

The evidence of stress markers in all age groups may indicate malnutrition due to seasonal shortages in the food supply or an early change in subsistence resources. Males appear to have been particularly affected as evidenced by early bone loss. Since the Moita population was in the later stages of the Mesolithic, it is expected that skeletal tissues might show evidence of any change in subsistence.

The high correlation between stature and the number of transverse lines in the cortical bone of the 12 individuals indicate that they were stressed by illness or seasonal food shortages severe enough to have had an effect on growth. Even in adults, appositional bone deposition (transverse lines) showed periodic stress. Evidence of physical trauma and physiological

stress in this small group of males and females under 40 years of age may indicate nutritional deprivation because of lower status within the group. They may have migrated into the area to take advantage of a wider range of subsistence resources.

Status may also be involved in the early loss of cortical thickness in males over age 45 since females were not affected by bone loss. Cortical thinning may have occurred before age 45-50 but there were no males representing the 40-45 year age group. Apparently the causative agent was specific to males and may have been associated with status. Consumption of alcohol produced from grain and its involvement with bone loss is uncertain since the Moita population were supposedly in the pre-agricultural phase of the transition from the Mesolithic to the Neolithic. Wild grain might have been gathered and utilized in the same manner as cultivated grain. Assuming alcohol was produced and intake was status-related, females may not have had contact with the beverage.

Another status-related male activity involves hunting. The most experienced and successful hunters might be expected to be males over 40 years of age. If liver was commonly eaten at the kill site hunters may have consumed an excess of vitamin A with resultant bone loss.

The males in the group with cortical transverse lines were under 40 years of age and would not be expected to show age related bone loss although several males did not have age assessments. If they were of lower status they might not have been permitted access to alcohol and may not have obtained

sufficient vitamin A from organ meats.

Even though there was no apparent cortical thinning in these males there was evidence of endosteal resorption in several males. This suggests that cortical thinning was just commencing. It also may suggest that they were gradually achieving status or acceptance and were integrating into the main group.

It is difficult to isolate a specific cause for cortical bone loss in males. The fact that the population at Moita was in the transition suggests that there may have been some utilization of grain. Whether the grain was gathered or obtained from experimentation with grain growing or in trade is unknown. There is also the possibility that premature aging, alcohol and excess ingestion of vitamin A were all involved.

The biological evidence in both the bones and teeth of all age groups in the Moita population suggests that problems with nutrition or illness were not of recent origin. It appears that the people living during the transitional phase may have had problems with food shortages because of seasonal fluctuations in the food supply or inadequate resources to provide for the needs of an increasing population. A change in subsistence strategies may have been necessary to overcome competition from increasing population numbers and insufficient food resources.

A larger sample might have provided more information since the small sample used in this research did not have equivalent sets of data for all individuals. An absence of one or more sets of values, especially age estimates, limited the amount of information obtained and affected the conclusions. As an example,

one male (L17) lacked an age assessment but endosteal bone loss and cortical thickness measurements indicated an age comparable to males over 40 with cortical thinning.

Indications of early bone loss in males and retention of thick cortices in females may have been a normal feature of this population. It may be possible to check the observations and conclusions in this research with other sites in Portugal. The neighboring sites of Cabeco da Amoreira and Cabeco da Arruda (see Lubell and Jackes 1985) might provide information that can be used to determine when grain was first cultivated, whether fluorescent labeling was present in the bone of either of these populations, and whether the health status was improved at the Neolithic site compared to those of the Mesolithic site at Moita. Research in this direction is required to understand more completely the health conditions that were present during the transition from hunting and gathering to food production.

TABLES

TABLE 1

MOITA DO SEBASTIÃO FEMORA

# ID	SIDE	SEX	CORTEX WIDTH (h)	NI	SINGH INDEX	ENDOSTEAL (2)	ENDOSTEAL (3)	TIME	DENSITY (g/cm^3)	SUTURE (cm)	LINES
#M0001	2	2	5.315	64	4	11.14	12.00	3.00	1.96	147	
#M0002	1	1	5.721			7.86	7.50	1.45	2.09		2
#M0003	1	1	6.261	58						166	
#M0003	2	1	6.549			7.83	8.00	2.00	1.67		
#M0004	2	1	5.925	67							
#M0006	2	1	5.041	67	6	11.00	10.25	3.00	2.52	161.5	
#M8007	2	2	6.192	59		8.50	9.75	2.15	2.46		
#M0008	2	1	4.595	58	5	10.60	10.75	3.00	2.25	158.5	
#M0009	2	1	6.375			11.67	10.50	3.00	1.51		
#M0010	2	2	7.452	65	6	7.50	9.00	3.30	2.12		
#M0011	1	1	7.983			8.13	8.00	6.30	2.32		
#M0013	2	1	6.621	57	6	6.43	7.75	7.00	2.00	163	7
#M0014	2	1	8.020	70	6	10.00	10.50	2.00	1.85		
#M0016	2	1	6.272	60	5			2.00	1.59	155	
#M0017	2	1	6.341	59	4	13.25	14.25	2.00	2.92	169	8
#M0018	1	1	7.261			9.75	9.25	3.50	2.18		
#M0018	2	1	6.841	67	4	5.83	6.50	5.00	2.06	167	8
#M0020	2	2	5.240	65	6	7.00	7.75	7.00	1.74	161	11
#M0020	1	2	5.233			8.33	9.00	1.20	1.70		
#M0025	2	1	7.314	70	3	11.50	11.50	2.00	2.87	157	12
#M1025	2	2	5.331	61	2	9.25	11.25	1.40	2.58		2
#M0030	1	1	5.641			7.83	8.00	2.00	2.52	156	
#M0032	2	1	6.549	56	6	8.17	8.75	2.45	1.86	156	
#M0035	2	2	5.667	59	5	7.25	7.50	1.30	1.67		
#M0037	2	1	5.224			8.60	8.75	0.45	2.58		
#M0040	1	2	5.729	62	3	10.67	11.25	6.30	1.86		10
#M0051	2	1	6.248	67	4						
#M0052	1	2	6.532			7.50	8.00	4.30	1.73		
#M0052	2	2	6.886	65	5						
#M0053	2	1	7.426	65	5	11.88	11.75	5.45	2.00	167	8
#M0054	2	2	5.131			9.57	10.00	1.00	2.94	144.5	
#M0057	2	1	6.230	66	6	6.13	6.50	8.15	1.57		9
#M0058	2	2	6.332	73	5	10.33	9.75	5.00	2.25	145	
#M000A	2	1	6.201	60		9.38	9.50	3.45	1.70	162	10
#M000C	2	1	5.292	52						156	
#M00CT	2	1	6.821	62		8.29	8.25	2.00	2.54	165	
#M00D2	2	0	5.121	61		9.67	9.00	1.10	1.21		5
#M00D7	2	2	6.429	70		9.00	8.75	1.00	1.63		

Data derived from analysis of the Moita femora.
Time: refers to the number of minutes to cut a section from a core.
Sex: 1 (male), 2 (female).
Side: 1 (right femur), 2 (left femur).
Cortex width (h): height of core in mm.
NI: Nordin Index
Lines: refers to number of transverse lines in thin sections.

TABLE 2

TOTAL THIN SECTION POROSITY IN COUNT 1

	Periosteal			Medial			Endosteal			T	P	M	E
	n	$\bar{X}$	sd	n	$\bar{X}$	sd	n	$\bar{X}$	sd	$\bar{X}$	%	%	%
L1	4	11.50	0.58	4	12.25	2.40	4	12.00	2.60	11.90	32.20	34.30	33.60
R2	7	9.10	0.90	8	9.10	0.64	7	7.90	3.50	8.70	34.90	34.90	30.30
L3	7	8.40	1.70	6	6.80	1.50	6	6.80	1.50	7.30	38.20	30.90	30.90
L6	4	12.75	3.86	7	12.40	2.70	8	12.00	2.20	12.40	34.30	33.40	32.30
L8007	8	7.13	1.36	8	6.63	1.06	8	7.88	1.25	7.11	32.95	30.64	36.41
L8	5	13.20	0.45	5	10.60	2.60	5	10.40	1.10	11.40	38.60	31.00	30.40
L9	7	14.30	0.49	7	11.40	1.70	6	10.30	1.60	12.00	39.00	31.70	28.60
L10	7	11.30	2.00	8	9.40	3.20	8	6.90	2.20	9.20	40.90	34.10	25.00
R11	6	14.20	2.60	7	9.90	1.80	7	8.90	2.30	11.00	43.00	30.00	27.00
L13	8	10.10	1.00	7	10.00	2.00	7	7.10	1.60	9.20	36.60	37.70	25.70
L14	6	8.20	2.30	7	9.30	2.40	7	10.60	1.60	9.40	29.20	33.10	37.70
L17	8	9.60	2.30	8	10.60	1.60	8	11.75	4.10	10.60	30.00	33.20	36.80
L18	7	11.40	2.70	6	10.20	1.80	5	5.80	1.60	9.10	41.60	37.20	21.20
R18	8	9.25	3.15	8	10.50	3.20	8	8.75	1.80	9.50	32.40	36.80	30.70
L20	7	13.70	2.20	8	10.90	3.40	7	8.00	2.60	10.90	42.00	33.40	24.50
R20	5	14.00	2.50	6	12.70	2.30	6	8.30	2.00	11.70	40.00	36.30	23.70
L25	5	9.80	0.84	5	9.40	2.40	4	11.50	1.70	10.20	31.90	30.60	37.50
L1025	8	9.40	2.30	8	11.25	1.60	8	7.75	3.10	9.50	33.10	39.60	27.30
R30	6	10.70	1.40	6	8.50	1.90	6	8.00	1.80	9.10	39.30	31.20	29.40
L32	3	11.70	2.10	7	10.00	2.50	7	9.40	2.40	10.40	37.60	32.20	30.20
L35	7	8.10	1.10	8	11.40	2.40	8	8.90	1.70	9.50	28.50	40.10	31.30
L37	6	13.50	1.90	6	12.00	1.90	5	8.40	2.50	11.30	39.80	35.40	24.80
R40	8	16.50	2.20	7	13.40	3.50	8	12.50	1.30	14.10	38.90	31.60	29.50
R52	7	10.60	1.70	6	10.20	1.30	6	8.30	1.00	9.70	36.40	35.05	28.50
L53	7	10.30	2.10	8	9.90	1.60	9	11.60	1.20	10.60	32.40	31.10	36.50
L54	7	9.60	1.70	7	10.00	2.40	6	8.70	1.90	9.40	33.90	35.30	30.70
L57	6	14.50	2.00	7	10.30	1.80	7	6.00	1.80	10.30	47.10	33.40	19.50
L58	6	12.00	1.10	8	11.75	2.25	6	10.00	2.10	11.25	35.60	34.80	29.70
LA	7	13.30	2.20	7	11.00	1.10	8	10.20	2.00	11.50	38.60	31.90	29.60
LCT	7	9.10	1.60	7	10.70	2.10	8	7.50	1.85	9.10	33.30	39.20	27.50
L0002	6	11.80	2.30	6	11.00	1.30	5	8.60	1.70	10.50	37.60	35.00	27.40
L0007	8	12.90	2.85	8	9.60	1.70	7	8.30	1.80	10.30	41.90	31.20	26.90

Data on the number of bone resorption spaces counted in 0.92 mm^2 fields across the width of each thin section of Moita bone.
Left (L) and right (R) femora.
n: number of 0.92 mm^2 fields counted.
sd: standard deviation.
$\bar{X}$: mean number of resorption spaces across n fields.,
T: total mean number of resorption spaces across the three areas P,M,E.
P: periosteal one-third.
M: medial one-third.
E: endosteal one-third.

TABLE 3

TOTAL THIN SECTION POROSITY IN COUNT 2

	Periosteal			Medial			Endosteal			T	P	M	E
	n	$\bar{x}$	sd	n	$\bar{x}$	sd	n	$\bar{x}$	sd	$\bar{x}$	%	%	%
L1	7	11.71	1.91	7	10.28	1.67	7	11.14	1.96	11.04	35.00	31.00	34.00
R2	9	10.22	2.44	8	11.00	2.12	7	7.86	1.81	9.69	35.00	38.00	27.00
L3	6	9.50	2.14	6	7.83	2.11	6	7.83	1.57	8.39	38.00	31.00	31.00
L6	8	12.13	1.96	8	12.25	2.44	9	11.00	1.25	11.79	34.00	35.00	31.00
L8007	8	8.75	1.64	8	7.25	2.22	8	8.50	2.69	8.17	36.00	30.00	35.00
L8	7	10.38	2.11	6	9.50	1.26	5	10.60	1.02	10.16	34.00	31.00	35.00
L9	8	12.00	2.49	7	13.00	2.00	6	10.00	1.91	11.67	34.00	37.00	29.00
L10	7	14.00	2.10	9	10.56	1.17	8	7.50	2.70	10.69	44.00	33.00	23.00
R11	9	11.56	2.22	9	9.33	2.05	8	8.13	3.02	9.67	40.00	32.00	28.00
L13	8	10.38	1.58	7	11.86	1.25	7	6.43	1.59	9.56	36.00	41.00	22.00
L14	7	14.43	2.38	7	11.57	1.29	7	10.00	1.69	12.00	40.00	32.00	28.00
L17	8	9.75	1.64	9	10.00	1.41	8	13.25	3.03	11.00	30.00	30.00	40.00
R18	8	10.38	2.60	8	12.00	2.87	8	9.75	1.56	10.71	32.00	37.00	30.00
L18	7	11.86	1.55	7	10.86	2.23	6	5.83	1.21	9.52	42.00	38.00	20.00
L20	8	18.25	2.17	8	9.50	4.36	8	7.00	2.55	11.58	53.00	27.00	20.00
R20	6	18.83	3.44	6	12.50	1.71	6	8.33	1.70	13.22	47.00	32.00	21.00
L25	5	9.60	1.20	5	11.20	2.23	4	11.50	0.86	10.77	30.00	35.00	36.00
L1025	8	10.00	2.35	8	10.25	3.86	8	9.25	2.86	9.83	34.00	35.00	31.00
R30	6	9.17	1.07	6	7.00	1.15	6	7.83	1.07	8.00	38.00	29.00	33.00
L32	3	10.67	1.70	4	9.00	1.41	6	8.17	1.86	9.28	38.00	32.00	29.00
L35	8	7.38	1.22	8	11.25	2.28	8	7.25	1.64	8.63	29.00	43.00	28.00
L37	6	14.00	2.52	6	10.17	2.41	2	8.60	2.94	10.92	43.00	31.00	26.00
R40	8	16.50	2.65	8	14.25	2.73	9	10.67	2.21	13.80	40.00	34.00	26.00
R52	7	9.57	1.59	7	8.00	1.51	6	7.50	0.96	8.36	38.00	32.00	30.00
L53	8	10.00	2.35	8	9.25	0.97	8	11.88	2.15	10.38	32.00	30.00	38.00
L54	8	10.38	2.96	8	11.00	2.50	7	9.57	0.90	10.32	34.00	36.00	31.00
L57	8	17.50	2.06	7	10.43	2.06	8	6.13	2.37	11.35	51.00	31.00	18.00
L58	6	11.17	1.67	8	10.25	1.56	6	10.33	3.09	10.58	35.00	32.00	33.00
LA	8	11.88	1.96	8	8.25	1.56	8	9.38	2.27	9.84	40.00	28.00	32.00
LCT	8	9.62	1.65	9	12.11	1.37	7	8.29	1.83	10.00	32.00	40.00	28.00
L0002	6	11.83	1.57	6	11.33	1.25	6	9.67	1.49	10.94	36.00	35.00	30.00
L0007	8	11.50	2.06	8	10.25	1.39	7	9.00	1.69	10.25	37.00	33.00	29.00

Data on the number of bone resorption spaces counted in 0.92 mm^2 fields across the width of each thin section of Moita bone.
Left (L) and right (R) femora.
n: number of 0.92 mm^2 fields counted.
sd: standard deviation.
$\bar{x}$: mean number of resorption spaces across n fields
T: total mean number of resorption spaces across the three areas P,M,E.
P: periosteal one-third.
M: medial one-third.
E: endosteal one-third.

TABLE 4

The mean number of resorption spaces per 0.92 mm^2 fields in count two over three cortical areas: individuals 57 and 35 compared.

	High L57	Low L35		High L57	Low L35
P	17.50	7.38	P	17.50	7.38
M	10.43	11.25	E	6.13	7.25
E	6.13	7.25			
χ^2 =	3.18		χ^2 =	2.216	
df =	2		df =	1	
P =	0.204		P =	0.137	

TABLE 5

Example of the data obtained in count two (PME) for the number of resorption spaces (porosity)
Individual: 57

Cortical area	No. of spaces $\bar{x}$	0.92 mm^2 fields n	Porosity %
P 1/3	17.50	8	51
M 1/3	10.43	7	31
E 1/3	6.13	8	18
Total	262 $\bar{x}$ = 11.39	23	100

TABLE 6a

Correlation Matrix among the three cortical areas (PME) in porosity counts one and two.

	P	M	E
P	0.682		
M		0.626	
E			0.880

TABLE 6b

The correlations between the mean number of resorption spaces (porosity) per 0.92 mm^2 among the three cortical areas, periosteal (P), mid-cortical (M), and endosteal (E) in two separate and independent counts.

	P	M	E		P	M	E
P		0.610	0.185	P		0.361	- 0.168
M			0.429	M			0.178
E				E			
	Count one				Count two		

TABLE 7

ANOVA SUMMARY TABLE FOR A COMPARISON OF MEANS

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F	Significance of F
Between (groups)	3	715.56	238.52	11.83	0.05
Within (groups)	15	302.38	20.16	---	
Total	18	1017.94	---	---	

REFERENCES CITED

Acsádi, G. and J. Nemeskéri
1970 History Of Human Life Span and Mortality. Budapest: Akademiai Kaidó.

Adams, P., G.T. Davies and P. Sweetnam
1970 Osteoporosis and the effects of aging on bone mass in elderly men and women. Quarterly Journal of Medicine 39:601-615.

Adams, C.F. and M. Richardson
1980 Source Book on Food and Nutrition. Chicago: Marquis Academic Media.

Ahlqvist,J. and O. Damsten
1969 A modification of Kerley's method for the microscopic determination of age in human bone. Journal of Forensic Sciences 14:205-213.

Albanese, A.A.
1977 Bone Loss: Causes, Detection and Therapy. New York: Alan R. Liss, Inc.

Alland, A.
1970 Adaptation in Cultural Evolution: An Approach to Medical Anthropology. New York: Columbia University Press.

Allison, M.J.
1984 Palaeopathology in Peruvian and Chilean populations. In: M.N. Cohen and G.J. Armelagos (eds.), Paleopathology at the Origins of Agriculture. Pp. 515-529. Toronto: Academic Press, Inc.

Alvioli, L.V.
1983 Osteoporosis. In: A. Peck (ed.), Bone and Mineral Research Annual 1. Pp. 280-318. New York: Elsevier Science Publishing Co.,Inc.

Anand, C.R. and H.M. Linkswiler
1974 Effect of protein intake on calcium balance of young men given 500 mgm calcium daily. Journal of Nutrition 104:695-700.

Angel, J.L.
1969 The bases of paleodemography. American Journal of Physical Anthropology 30:427-437.

Armelagos, G.J., J.H. Mielke, K.H. Owen, D.P. VanGerven, J.R. Dewey, and P.E. Mahler
1972 Bone growth and development in prehistoric

populations from Sudanese Nubia. Journal of Human Evolution 1:89-119.

Arnold, J.S.
1970 Focal excessive endosteal resorption in aging and senile osteoporosis. In: U.S. Barzel (ed.), Osteoporosis. Pp. 80-100. New York: Grune and Stratton.

Arnold, J.S., M.H. Bartley, S.A. Tont, and D.P. Jenkins
1966 Skeletal changes in aging and disease. Clinical Orthopaedics 49:17-38.

Ascenzi, A.
1955 Some histochemical properties of the organic substance in Neandertalian bone. American Journal of Physical Anthropology 13(4):557-566.

Ascenzi, A.
1983 Microscopic dissection and isolation of bone constituents. In: A.S. Kunin and D.J. Simmons (eds.), Skeletal Research. Pp. 185-236. New York: Academic Press, Inc.

Atkinson, P.J., J.A. Weatherell, and S.M. Weidman
1962 Changes in bone density of the human femoral cortex with age. Journal of Bone and Joint Surgery 44B:496-502.

Atkinson, P.J.
1964 Quantitative analysis of osteoporosis in cortical bone. Nature 201:373-375.

Atkinson, P.J., and J.A. Weatherell
1967 Variation in the density of the femoral diaphysis with age. Journal of Bone and Joint Surgery 49B:781-788.

Atkinson, P.J. and C. Woodhead
1973 The development of osteoporosis. Clinical Orthopaedics 90:217-228.

Baker, P.T. and J. L. Angel
1965 Old age changes in bone density: sex, and race factors in the United States. Human Biology 37:104-121.

Baker, P.T. and M.A. Little
1965 Bone density changes with age, altitude, sex and race factors in Peruvians. Human Biology 37:122-136.

Balthazard, V. and F. Lebrun
1911 Les canaux de Havers de l'os humain aux différents

âges. Annales d'Hygiène Publique Médecine Légal 15:144.

Bancroft, J.D. and A. Stevens (eds.)
1977 Theory and Practice of Histological Techniques. Edinburgh: Churchill-Livingstone.

Barer, M. and J. Jowsey.
1967 Bone formation and resorption in normal human rib. A study of persons from 11 to 88 years of age. Clinical Orthopaedics 52: 241-247.

Barnett, E. and B.E.C. Nordin
1960 The radiological diagnosis of osteoporosis: a new approach. Clinical Radiology 11:166-174.

Barnicot, N. A. and S.P. Datta
1972 Vitamin A and bone. In: G.H. Bourne (ed.), The Biochemistry and Physiology of Bone. Pp.197-229. New York: Academic Press, Inc.

Bartley, M.H. and J.S. Arnold
1967 Sex differences in human skeletal involution. Nature 214:908-909.

Bassett, E.J., M.S. Keith, G.J. Armelagos and D.L. Martin
1980 Tetracycline-labeled human bone from ancient Sudanese Nubia (A.D. 350). Science 209:1532-1534.

Bassett, E.S.
1981 Tetracycline staining in a pre-historic population from Sudanese Nubia. Bulletin et Mémoire de la Société d'Anthropologie de Paris, t.8, série XIII, pp. 321-322.

Beattie, O.
1985 Personal communication. University of Alberta: Professor, Department of Anthropology.

Beddoe, A.H.
1977 Measurement of the microscopic structure of bone. Physics in Medicine and Biology 22(1):298-308.

Bender, M.M., D.A. Baerreis and R.L. Steventon
1981 Further light on carbon isotopes and Hopewell agriculture. American Antiquity 46:346-353.

Bergot, C. and J.P. Bocquet
1976 Étude systématique en fonction de l'age de l'os spongieux et de l'os cortical de l'humérus et du fémur. Bulletin et Mémoire de la Société d'Anthropologie de Paris, t.3, série XIII, pp. 215-242.

Bigalke, E.H.
1973 The exploitation of shellfish by coastal tribesmen of the Transkei. Annals of the Cape Provincial Museums (Natural History) 9(9):159-175.

Binford, L.R.
1968 Post-pleistocene adaptations. In: S.R. Binford and L.R. Binford (eds.), New Perspectives in Archaeology. Pp. 313-341. Chicago: Aldine.

Blakey, M.L.
1981 An Analysis of hypoplasia and hypocalcification in deciduous dentition from Dickson Mound. In: D.B. Martin and M.P. Bumstead (eds.), Biocultural Adaptation: Comprehensive Approaches to Skeletal Analysis. Pp. 24-34. Research Reports Number 20. Department of Anthropology. University of Massachusetts, Amherst.

Blakely, R.L. and L.A.Beck
1981 Trace elements, nutritional status, and social stratification at Etowah, Georgia. Annals of the New York Academy of Science 376:417-431.

Bloom, W. and D.W. Fawcett
1975 A Textbook of Histology. Toronto: W.B. Saunders Company.

Blumberg, J.M. and E.R. Kerley
1965 Discussion: a critical consideration of roentgenology and microscopy in palaeopathology. In: S. Jarcho (ed.), Human Palaeopathology. Pp. 150-170. London: Yale University Press.

Bocquet-Appel, J. and C. Masset
1982 Farewell to paleodemography. Journal of Human Evolution 11:321-333.

Boutton, T.W., P.D. Klein, M.J. Lynott, J.E. Price and L.L. Tieszen
1984 Stable carbon isotope ratios as indicators of prehistoric human diet. In: J.R. Turnlund and P.E. Johnson (eds.), Stable Isotopes in Nutrition. Pp. 191-204. Washington,D.C.: American Chemical Society.

Bouvier,M. and D.H. Ubelaker
1977 A comparison of two methods for the microscopic determination of age at death. American Journal of Physical Anthropology 46:391-394.

Budge, E.W.
1978 Herb-Doctors and Physicians in the Ancient World. Chicago: Ares Publishers Inc.

Buikstra, J.E. and D.C. Cook
1980 Palaeopathology: an American account. Annual Review of Anthropology 9:433-470.

Buikstra, J. E.
1984 The lower Illinois river region: a prehistoric context for the study of ancient diet and health. In: M.N. Cohen and G.J. Armelagos (eds.), Palaeopathology at the Origins of Agriculture. Pp. 215-234. Toronto: Academic Press, Inc.

Buikstra J.E. and L.W. Konigsberg
1985 Paleodemography: critiques and controversies. American Anthropologist 87:316-333.

Bullamore, J.R., J.C. Gallagher, R. Wilkinson, B.E.C. Nordin and D.H. Marshall
1970 Effect of age on calcium absorption. Lancet 2:535-537.

Campbell, J.A.
1973 Nutritional surveillance in Canada. In: D. Hollingsworth and M. Russel (eds.), Nutritional Problems in a Changing World. Pp.83-89. London: Applied Science Publishers Ltd.

Carlson, D.S., G.J. Armelagos, and D.P. Van Gerven
1976 Patterns of age-related cortical bone loss (osteoporosis) within the femoral diaphysis. Human Biology 48(2):295-314.

Cassidy, C.M.
1980 Nutrition and health in agriculturists and hunter-gatherers . In: N.W. Jerome, R.F. Kandel and G.H. Pelto (eds.), Nutritional Anthropology: Contemporary Approaches to Diet and Culture. Pp. 117-145. New York: Redgrave Publishing Company.

Chandler, R.F., L. Freeman and S.N. Hooper
1979 Herbal Remedies of the Maritime Indians. Journal of Ethnopharmacology 1:49-68.

Childe, V.G.
1936 Man Makes Himself. London: Watts.

Coale, J.A.
1974 The history of human population. Scientific American 231:40-51.

Cockburn, T.A.
1971 Infectious diseases in ancient populations. Current Anthropology 12:45-62.

Cohen, M.N.
1977 The Food Crisis in Prehistory: Overpopulation and the Origins of Agriculture. New Haven: Yale University Press.

Cook,H.C.
1974 Manual of Histological Demonstration Techniques. Toronto:Butterworth Co. (Canada) Ltd.

Cook, D.C.
1979 Subsistence base and health in prehistoric Illinois valley: evidence from the human skeleton. Medical Anthropology 3:109-124.

Cook, D.C.
1984 Subsistance and health in the lower Illinois valley: osteological evidence. In: M.N. Cohen and G.J. Armelagos (eds.), Palaeopathology at the Origins of Agriculture. Pp. 235-269. New York: Academic Press, Inc.

Coon, C.S.
1976 The Hunting Peoples. Dallas, Pennsylvania: Offset Paperback Mfrs.,Inc.

Currey, J.D.
1964 Some effects of aging in human Haversian systems. Journal of Anatomy (London) 98:69-75.

Davis, C.E. and J. Anandan
1970 The evolution of R factor. The New England Journal of Medicine 282(3):117-122.

De Luca, H.F.
1980 The control of calcium and phosphorous metabolism by the vitamin D endocrine system. Annals of the New York Academy of Science 355:1-17.

DeLuca, H.F.
1982 Vitamin D. In: A. Neuberger and T.H. Jukes (eds.), Human Nutrition. Pp. 141-184. Englewood, New Jersey: Jack K. Burgess, Inc.

DeNiro, M.J. and S. Epstein
1981 Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341-351.

Dequeker, J.
1972 Bone Loss in Normal and Pathological Conditions. Leuven, Belgium: Leuven University Press.

Dequeker, J., Y.S. Roh and K. Gautama
1976 Evaluation of the femoral trabecular pattern grading

system, its value in spinal osteoporosis and femoral neck fracture. In: Z.F.G. Jaworski (ed.), Proceedings of the First Workshop on Bone Morphometry. Ottawa, March 28-31, 1973. Pp. 89-93. Ottawa: University of Ottawa Press.

Deslypere, P. and H. Baert
1958 Assessment of age by the measurement of the Haversian canals of human bones: a critical study of the Balthazard and Lebrun method. Journal of Forensic Medicine 5(4):195-199.

Dewey, J.R., G.J. Armelagos, and M.H. Bartley
1969 Femoral cortical involusion in three Nubian archaeological populations. Human Biology 41:13-28.

Drury, R.A.B. and E.A. Wallington (eds.)
1980 Carlton's Histological Techniques. Toronto:Oxford University Press.

Duncan, H.
1976 Cortical porosis: a morphological evaluation. In: Z.F.G. Jaworski (ed.), Proceedings of the First Workshop on Bone Morphometry. Ottawa, March 28-31, 1973. Pp. 78-83. Ottawa: University of Ottawa Press.

Eaton, S.B., and M. Konner
1985 Paleolithic Nutrition. The New England Journal of Medicine 312 (5):283-289.

Elvin-Lewis, Memory
1979 Empirical rationale for teeth cleaning plant selection. Medical Anthropology 3(2):431-458.

Ericksen, M.F.
1980 Patterns of microscopic bone remodeling in three aboriginal American populations. In: D.L. Browman (ed.), Early Native Americans. Pp. 239-270. The Hague: Mouton.

Ericksen, M.F.
1976 Cortical bone loss with age in three native American populations. American Journal of Physical Anthropology 45:443-452.

Ericksen, M.F.
1982 Aging changes in the proximal femoral cortex. American Journal of Physical Anthropology 59:121-130.

Exton-Smith, A.N., H.M.Hodkinson, and B.E. Stanton
1966 Nutrition and metabolic bone disease in old age. Lancet 2:199-1001.

Ferembach, D.
1972 Le gisement mésolithique de Moita do Sebastião, Muge, Portugal, 11. Anthropologie. Lisboa: Instituto de Alta Cultura.

Flannery, K.V.
1965 The ecology of early food production in Mesopotamia. Science 147:1247-1256.

Frank, E.E.
1981 Femoral bone mineral content and cortical thickness in Aleut and Eskimo adults. In: D.L. Martin and M.P. Bumstead (eds.), Biocultural Adaptations: Comprehensive Approaches to Skeletal Analysis. Pp. 78-83. Research Reports No. 20. Department of Anthropology. University of Massachusetts, Amherst.

Frost, H.M.
1958 Preparation of thin undecalcified bone sections by the rapid manual method. Stain Technology 33:272-276.

Frost, H.M.
1964 Mathematical Elements of Lamellar Bone Remodeling. Springfield, Illinois: Charles C. Thomas.

Frost, H.M.
1969 Tetracycline-based histological analysis of bone remodeling. Calcified Tissue Research 3:211-237.

Frost, H.M.
1963 Bone Remodeling Dynamics. Springfield, Illinois: Charles C. Thomas.

Frost, H.M.
1973 Bone Remodeling And its Relationship to Metabolic Bone Disease. Springfield, Illinois: Charles C. Thomas.

Galigher, A.E. and E.N. Kozloff
1971 Essentials of Practical Microtechnique. Philadelphia: Lee and Febiger.

Gallagher, J.C., B.L. Riggs, J. Eisman, A. Hamstra, S.B. Arnaud, and H.F. DeLuca
1979 Intestinal calcium absorption and serum vitamin D Metabolites in normal subjects and osteoporotic patients. Journal of Clinical Investigation 64: 729-736.

Ganguly, J. and S.K. Murthy
1967 Biogenesis of vitamin A and carotene. In: W.H. Sebrell Jr. and R.S. Harris (eds.), The Vitamins 1: 125-152. New York: Academic Press, Inc.

Garn, S.M. and R.S. Baby
1969 Bilateral symmetry in finer lines of increased density. American Journal of Physical Anthropology 31:89-92.

Garn, S.M.
1970 The Earlier Gain and Later Loss of Cortical Bone. Springfield, Illinois: Charles C. Thomas.

Garn, S.M.
1972 The course of bone gain and the phases of bone loss. Orthopedic Clinics of North America 3(3):503-520.

Garn, S.M. and M.A. Solomon
1981 Do the obese have better bones? Ecology of Food and Nutrition 10: 195-197.

Garn, S.M.
1981 The phenomenon of bone formation and bone loss. In: H.F. DeLuca, H.M. Frost, W.S.S. Gee, C.C. Johnston Jr., and A.M. Parfitt (eds.), Osteoporosis. Pp. 3-16. Baltimore: University Park Press.

Gilbert, B.M. and T.W. McKern
1973 A method for aging the female os pubis. American Journal of Physical Anthropology 38:31-38.

Gilbert, R.
1975 Trace Element Analysis of Three Skeletal Amerindian Populations at Dickson Mounds. Unpublished Ph.D. dissertation, University of Massachusetts, Amherst.

Gilbert, R.I. Jr.
1985 Stress, paleonutrition, and trace elements. In: R.I. Gilbert, Jr. and J.H. Mielke (eds.), The Analysis of Prehistoric Diets. Pp. 339-358. New York: Academic Press, Inc.

Gindhart, Schwager P.
1969 The frequency of appearance of transverse lines in the tibia in relation to childhood illnesses. American Journal of Physical Anthropology 31: 17-23.

Goldsmith, N.F.
1973 Normative data from the osteoporotic prevalence survey, Oakland, California, 1969-1970. Bone mineral at the distal radius: variation with age, sex, skin color, and exposure to oral contraceptives and exogenous hormones; relation to aortic calcification, osteoporosis, and hearing loss. In: R.B. Mazess (ed.), Proceedings of the International Conference on Bone Mineral Measurement. Chicago, Illinois, October 12-13, 1973. Pp.239-266. United States Department

of Health, Education, and Welfare. DHEW Publication No. (NIH) 75-683. Washington, D.C.: United States Government Printing Office.

Goodman, A.H. and G.A. Clark
1981 Harris lines as indicators of stress in prehistoric Illinois populations. In: D.B. Martin and M.P. Bumstead (eds.), Biocultural Adaptation; Comprehensive Approaches to Skeletal Analysis. Pp. 35-46. Research Reports No. 20. Department of Anthropology. University of Massachusetts, Amherst.

Goodman, J.J.
1985 Fermentation and mutational development of the tetracyclines. In: J.J. Hlavka and J.H. Boothe (eds.), The Tetracyclines 78:5-57. New York: Springer-Verlag.

Graf, W.
1949 Preserved histological structures in Egyptian mummy tissues and ancient Swedish skeletons. Acta Anatomica 8: 236-350.

Guthrie, H.A.
1975 Introductory Nutrition. Saint Louis: The C.V. Mosby Company.

Guyton, A.C.
1976 Textbook of Medical Physiology. Toronto: W.B. Saunders Company.

Hahn, T.J., V.C. Boisseau, and L.V. Alvioli
1974 The effect of chronic corticosteroid administration on diaphyseal and metaphyseal bone mass. Journal of Clinical Endocrine Metabolism 39:274-282.

Hall, B.K.
1978 Developmental and Cellular Skeletal Biology. New York: Academic Press.

Hancox, N.M.
1972 Biology of Bone. London: Cambridge University Press.

Hatch, J.W., P.S. Willey, and E.E. Hunt Jr.
1983 Indicators of status-related stress in Dallas society: transverse lines and cortical thickness in long bones. Midcontinental Journal of Archaeology 8(1): 49-71.

Henfrey, A. 1819-1859
1852 The Vegetation of Europe. London: John Van Voorst, Paternoster Row; reprint ed., New York: Arno Press Inc. F.N. Egerton 111, 1977.

Hummert, J.R. and D.P. Van Gerven
1982 Tetracycline-labeled human bone from a medieval population in Nubias' Batn El Hajar (550-1450 A.D.). Human Biology 54(2):355-371.

Hunt, E.E. and J.W. Hatch
1981 The estimation of age at death and ages of formation of transverse lines from measurements of human long bones. American Journal of Physical Anthropology 54:461-469.

Huss-Ashmore, R., A.H. Goodman, and G.J. Armelagos
1982 Nutritional inference from paleopathology. In: M.B. Schiffer (ed.), Advances in Archaeological Method and Theory. Pp. 395-474. New York: Academic Press, Inc.

Huss-Ashmore, R.
1981 Bone growth and remodeling as a measure of nutritional stress. In: D.L. Martin and M.P. Bumstead (eds.), Biocultural Adaptation: Comprehensive Approaches to Skeletal Analysis. Pp. 84-95 Research Reports No. 20. Department of Anthropology. Amherst: University of Massachusetts.

Ibsen, K.H., M.R. Urist, R.E. Sognaes, and J.Keen.
1965 Differences among tetracyclines with respect to the staining of teeth. Journal of Pediatrics 67:459-462.

Irving, J.T.
1973 Calcium and Phosphorus Metabolism. New York: Academic Press, Inc.

Isaac, E.
1970 Geography of Domestication. Englewood Cliffs, N.J.: Prentice-Hall Inc.

Jackes, M.K.
1985 Personal communication. University of Alberta: Associate Research Professor, Department of Anthropology.

Jackes, M.K. and D. Lubell
nd Where are the old folks? Manuscript in preparation for submission to M.S. Schiffer (ed.), Advances in Archaeological Method and Theory.

Jackes, M.
Osteological and palaeodemographic studies. In: Lubell, D., R. de Ceunynck, C. Devereux, A. Gautier, M. Jackes, C. Meiklejohn, P. Sheppard, and C. Schentag.
1984 Progress Report on Research Operating Grant 410-84-0030 for the period May 30, 1984 to January 31, 1985 inclusive. Manuscript on file in Edmonton, Winnipeg,

Ottawa, Gent, Lisbon, Evora, and Setubal.

Jackson, F.L.
1986 Personal communication. Department of Medical Microbiology and Infectious Diseases, University of Alberta.

Jaworski, Z.F.G.
1981 The quantum concept of bone remodeling in adults. In: H.F.DeLuca, H.M. Frost, W.S.S. Jee, C.C.Johnston Jr., and A.M. Parfitt (eds.), Osteoporosis: Recent Advances in Pathogenesis and Treatment. Pp. 103-113. Baltimore: University Park Press.

Jaworski, Z.F.G.
1983 Bone histomorphometry: outline of theory and practise. In: A.S. Kunin and D.J. Simmons (eds.), Skeletal Research: An Experimental Approach. Pp. 238-276. New York: Academic Press, Inc.

Jelliffe, D.B., J. Woodburn, F.J. Bennett, and E.F.P. Jelliffe
1962 The children of the Hadza hunters. Journal of Pediatrics 60:907-913.

Jette, M.
1976 Photon absorptiometry: the Cameron-Sorenson Method and its application to bone turnover studies. In: Z.F.G. Jaworski (ed.), Proceedings of The First Workshop on Bone Morphometry. Ottawa, March 28-31, 1973. Pp. 118-124. Ottawa: University of Ottawa Press.

Johnston, C.C. Jr., J.A. Norton Jr., R.A. Khairi, and C.Longcope
1979 Age-related bone loss. In: U.S. Barzel (ed.), Osteoporosis 11. Pp. 91-100. New York: Grune and Stratton.

Johnston, C.C. Jr., S.L. Hui, P. Niske, J.A. Norton Jr., and S. Epstein
1980 Bone mass at maturity and subsequent rates of loss as determinants of osteoporosis. In: H.F. DeLuca, H.M. Frost, W.S.S. Jee, C.C. Johnston Jr., and A.M. Parfitt (eds.), Osteoporosis: Recent Advances in Pathogenesis and Treatment. Pp. 285-291. Baltimore: University Park Press.

Jowsey, J.
1960 Age changes in Human bone. Clinical Orthopaedics 17:210-218.

Jowsey, J., P.J. Kelly, L.B. Riggs, A.J. Bianco Jr., D.A. Scholz, and J. Gershon-Cohen

1965 Quantitative microradiographic studies of normal and osteoporotic bone. Journal of Bone and Joint Surgery 47-A (4):785-806.

Jowsey, J. and G. Gordon
1971 Bone turnover and osteoporosis. In: G.H. Bourne (ed.), The Biochemistry and Physiology of Bone. Pp. 202-239. New York: Academic Press, Inc.

Kerley, E.R.
1965 The microscopic determination of age in human bone. American Journal of Physical Anthropology 23:149-163.

Kerley, E.R. and D.H. Ubelaker
1978 Revisions in the microscopic method of estimating age at death in human cortical bone. American Journal of Physical Anthropology 49:545-546.

Kühl,I.
1980 Harris´s lines and their occurrence also in the bones of prehistoric cremations. Ossa 7:129-171.

Kumar, R., W.R. Cohen, and F.H. Epstein
1980 Vitamin D and calcium hormones in pregnancy. New England Journal of Medicine 302(20): 1143-1145.

Krantz, G.E., R.R. Colwell, and E. Lovelace
1969 Vibrio parahaemolyticus from the blue crab Callinectes Sapidus in Chesapeake Bay. Science 164:1286-1287.

Kurzawski, V.M.
1983 Mise au Point sur les méthodes de détermination de l´âge par voie histologique osseuse. Bulletin et Mémoire de la Société d´Anthropologie de Paris t.10, série XIII:413-424.

Lacroix, P.
1971 The internal remodeling of bones. In: G.H. Bourne (ed.), The Biochemistry and Physiology of Bone. Pp. 119-144. New York: Academic Press, Inc.

Lambert, J.B., S.M. Vlasak, A.C.Thometz, and J.E. Buikstra
1982 A comparative study of the chemical analysis of ribs and femurs in Woodland populations. American Journal of Physical Anthropology 59:289-294.

Laughlin, W.S., A.B. Harper, and D.D. Thompson
1979 New approaches to the pre-and post contact history of Arctic peoples. American Journal of Physical Anthropology 51:579-587.

Lazenby, R.A.
1984 Inherent deficiencies in cortical bone

microstructural age estimation techniques. Ossa 9-11:95-103.

Lee, R.B.
1979 The !Kung San. New York: Cambridge University Press.

Lentacker, A.
1985 Archaeozoology of Medo Tojeiro and Samouqueira. Manuscript on file in Edmonton and Gent.

Lofgren, C.G., K.A. Omnell, and M.U. Nylen
1968 Effect of intraperitoneal injection of tetracycline hydrochloride and oxytetracycline on forming enamel of rat incisors. Calcified Tissue Research 2:145-156.

Lubell, D., M. Jackes, and C. Meiklejohn
1986a Archaeology and human biology of the Mesolithic-Neolithic transition in southern Portugal: a preliminary report. In: C. Bonsall (ed.), Proceedings of the 111 International Mesolithic Symposium, April 1985. Edinburgh: University of Edinburgh, Department of Archaeology (in press).

Lubell, D., M. Jackes, H. Schwarcz, and C. Meiklejohn
1986b New radiocarbon dates for Moita do Sebastião. Arqueologia 14 (in press).

Lubell, D., R. de Ceunynck, C. Devereux, A. Gautier, M. Jackes, C. Meiklejohn, P. Sheppard, and C. Schentag.
1984 Progress Report on Research Operating Grant 410-84-0030 for the period May 30, 1984 to January 31, 1985 inclusive. Manuscript on file in Edmonton, Winnipeg, Ottawa, Gent, Lisbon, Evora, and Setubal.

Lubell, D. and Jackes, M.
1985 Mesolithic-Neolithic continuity: evidence from chronology and human biology. In: M. Ramos (ed.), Actas do 1 Reunião do Quaternario Iberico. Pp. 113-133. Lisboa.

Maat, G.J.R.
1984 Dating and rating of Harris's lines. American Journal of Physical Anthropology 63:291-299.

Maddock, K.
1972 The Australian Aborigines. London:Allen Lane, Penguin Press.

Mallegni, F., R. Mariani-Costantini, G. Fornaciari, E.T. Longo, G. Giacobini and A.M. Radmilli
1983 New European fossil hominid material from an acheulian site near Rome (Castel Di Guido). American Journal of Physical Anthropology 62:263-274.

Manolagos, S.C., D.C. Anderson and R. Lindsay
1979 Adrenal steroids and the development of osteoporosis in oophorectomized women. Lancet 2:597-600.

Marshall, W.A.
1968 Problems in relating the presence of transverse lines in the radius to the occurrence of disease. In: D.R. Brothwell (ed.), The Skeletal Biology of Earlier Human Populations. Pp. 245-261. Toronto: Pergamon Press.

Martin, D.L., and G.J. Armelagos
1979 Morphometrics of compact bone: an example from Sudanese Nubia. American Journal of Physical Anthropology 51:571-578.

Martin, D.L.
1981 Microstructural analysis: possibilities for skeletal analysis. In: D.L. Martin, and M.P. Bumstead (eds.), Biocultural Adaptations: Comprehensive Approaches to Skeletal Analysis. Pp. 96-107. Research Reports No. 20. Department of Anthropology. University of Massachusetts, Amherst.

Martin, D.L., G.J. Armelagos, J.H. Mielke, and R.S. Meindl
1981 Bone loss and dietary stress in an adult skeletal population from Sudanese Nubia. Bulletin et Mémoire de la Société d'Anthropologie de Paris t.8, série XIII , pp. 307-319.

Martin, D., G.J. Armelagos, A.H. Goodman, and D.P. Van Gerven
1984 The effects of socioeconomic change in prehistoric Africa: Sudanese Nubia as a case study. In: M.N. Cohen and G.J. Armelagos (eds.), Palaeopathology at the Origins of Agriculture. Pp. 193-214. New York: Academic Press, Inc.

Martin, D.L., A.H. Goodman and G.J. Armelagos,
1985 Skeletal pathologies as indicators of quality and quantity of diet. In: J.Mielke and R. Gilbert (eds.), The Analysis of Prehistoric Diet. Pp. 227-279. New York: Academic Press, Inc.

Mathews, J.L.
1980 Bone structure and ultrastructure. In: M.R. Urist (ed.), Fundamental and Clinical Bone Physiology. Pp. 4-44. Toronto: J.P. Lippincott Company.

Mausner, J.S. and A.K. Bahn
1974 Epidemiology. Toronto: W.B. Saunders Company.

Mazess, R.B. and R. Jones
1972 Weight and density of Sadlermiut Eskimo long bones. Human Biology 44(3): 537-548.

Mazess, R.B. and J.R. Cameron
1973 Bone mineral content in normal U.S. Whites. In: R.B. Mazess (ed.), Proceedings of the International Conference on Bone Mineral Measurement. October 12-13, 1973. Chicago, Illinois. Department of Health, Education, and Welfare. DHEW Publication No. (NIH) 75-683. Pp. 28-238. Washington, D.C.: United States Government Printing Office.

Mazess, R.B. and W.E. Mather
1974 Bone mineral content of North Alaskan Eskimos. American Journal of Clinical Nutrition 27:916-925.

Mazess, R.B. and .E. Mather
1975 Bone mineral content in Canadian Eskimos. Human Biology 47:45-63.

Mazess, R.B.
1980 Noninvasive measurement of local bone. In: H.F. DeLuca, H.M. Frost, W.S.S. Jee, C.C. Johnston Jr., and A.M. Parfitt (eds.), Osteoporosis: Recent Advances in Pathogenesis and Treatment. Pp. 25-36. Baltimore: University Park Press.

Mazess, R.B.
1983 Noninvasive bone measurements. In: A.S. Kunin and D.J. Simmons (eds.), Skeletal Research. Pp. 277-343. Toronto: Academic Press, Inc.

McElroy, A. and P.K. Townsend
1979 Medical Anthropology in Ecological Perspective. North Scituate, Massachusetts: Duxbury Press.

McHenry, H.
1968 Transverse lines in long bones of prehistoric California Indians. American Journal of Physical Anthropology 29:1-18.

McKern, T.W. and T.D. Stewart
1957 Skeletal age changes in young American males. Headquarters, Quartermaster Research and Development Command, Technical Report EP-45. Natick, Massachusetts.

McLaren, D.
1967 Effects of vitamin A deficiency in man. In: W.H. Sebrell, Jr. and R.S. Harris (eds.), The Vitamins. Pp. 267-280. New York: Academic Press.

McQueen, C.M., I.B. Monk, P.W. Horton, and A.D. Smith
1972 Preparation of Undecalcified bone sections for auto- and microradiography. Calcified Tissue Research 10:23-30.

Meehan, B.
1982 Shell Bed To Shell Midden. Canberra: Australian Institute of Aboriginal Studies.

Meiklejohn, C.M.
1986 Radiocarbon dating of human skeletal material from two sites in Portugal. Mesolithic Miscellany 7(2):4-6.

Meiklejohn, C., C. Pardoe, and D. Lubell
1979 The adult skeleton from the Capsian site of Aïn Misteheyia, Algeria. Journal of Human Evolution 8:411-426.

Meindl, R.S., C.O. Lovejoy, and R.P. Mensforth
1983 Skeletal age at death: accuracy of determination and implications for human demography. Human Biology 55(1); 73-87.

Mendenhall, W., J.T. McClave and M. Ramey
1977 Statistics for Psychology. North Scituate, Massachusetts: Duxbury Press.

Mielke, J.H., G.J. Armelagos, and D.P. Van Gerven
1972 Trabecular involution in femoral heads of a prehistoric (X-Group) population from Sudanese Nubia. American Journal of Physical Anthropology 36:39-44.

Milch, R.A., D.P. Rall, and J.E. Tobie
1958 Fluorescence of tetracycline antibiotics in bone. Journal of Bone and Joint Surgery 40-A(4):897-910.

Mitscher, L.A.
1978 The Chemistry of the Tetracycline Antibiotics. New York: Marcel Dekker, Inc.

Montoye, H.J., J.F. McCabe, H.L. Metzner, and S.M. Garn
1976 Physical activity and bone density. Human Biology 48(3): 599-610.

Moore, T.
1967 Effects of vitamin A deficiency in animals. In: W.H. Sebrell Jr., and R.S. Harris (eds.), The Vitamins. Pp. 280-293. New York: Academic Press.

Myers, H.M.
1968 Alizarin and Tetracycline binding by bone mineral. American Journal of Physical Anthropology 29:179-182.

Nairn, R.C.
1976 Fluorescent Protein Tracing. New York: Churchill Livingstone.

Navia, J.M. and S.S.Harris
1980 Vitamin A influence on calciuum metabolism and calcification. Annals of the New York Academy of Science 355:45-57.

Newcomb, W.W. Jr.
1974 North American Indians: An Anthropological Perspective. Santa Monica, California: Goodyear Publishing Company, Inc.

Newell, R.R., T.S. Constandse-Westermann, and C. Meiklejohn
1979 The skeletal remains of Mesolithic man in Western Europe: an evaluative catalogue. Journal of Human Evolution 8(1):1-233.

Nickens, P.R.
1976 Stature reduction as an adaptive response to food production in Mesoamerica. Journal of Archaeological Science 3:31-41.

Nilsson, B.O.E. and N.E. Westlin
1971 Bone density in athletes. Clinical Orthopaedics 77:179-182.

Nonomura, E., M. Okomoto, S. Sobue and Y. Moriwaki
1977 X-ray microbeam diffraction analysis on a tooth discolored by tetracycline. Journal of Dental Research 56:447-450.

Nordin, B.E.C.
1966 International patterns of osteoporosis. Clinical Orthopaedics 45:17-30.

Nordin, B.E.C., M.M. Young, L. Bulusu, and A. Horsman
1970 Osteoporosis re-examined. In: U.S. Barzel (ed.), Osteoporosis. Pp. 47-67. New York: Grune and Stratton.

Nordin, B.E.C.
1973 Metabolic Bone and Stone Disease. London: Churchill Livingstone.

Norman, A.W.
1980 Bone and the vitamin D endocrine system. In: M.R. Urist (ed.), Fundamental and Clinical Bone Physiology. Pp. 242-267. Toronto: J.P. Lippincott Company.

Ogden, J.A.
1980 Chondro-osseus development and growth. In: M.R. Urist (ed.), Fundamental and Clinical Bone Physiology. Pp. 108-171. Toronto:J.P. Lippincott Company.

Oomen, H.A.P.C.
1976 Vitamin A deficiency, xeropthalmia and blindness. In: D.M. Hegsted, C.O. Chichester, W.J. Darby, K.W. McNutt, R.M. Stalvey, and E.H. Stotz (eds.), Present Knowledge in Nutrition. Pp. 73-81. New York: The Nutrition Foundation, Inc.

Ortner, D.J.
1970 The effects of aging and disease on the micromorphology of human compact bone. Unpublished Ph.D. dissertation, University of Kansas, Lawrence, Kansas.

Ortner, D.J.
1975 Aging effects on osteon remodeling. Calcified Tissue Research 18:27-36.

Ortner, D.J. and W.G.J. Putschar
1981 Identification of Pathological Conditions in Human Skeletal Remains. Smithsonian Contributions to Anthropology No.28.

Oswalt, W.H.
1982 This Land Was Theirs: A Study of North American Indians. New York: John Wiley & Sons.

Owen, G.M. and A.L. Owen
1982 Nutritional status of North Americans. In: A. Neuberger and T.H. Jukes (eds.), Human Nutrition. Pp. 73-86. Englewood, New Jersey: Jack K. Burgess, Inc.

Parfitt, A.M., C. Mathews, D.R.B. Frame, M. Kleerkoper, and A.R. Villanueva
1981b Impaired osteoblast function in metabolic bone disease. In: H.F. DeLuca, H.M. Frost, W.S.S. Jee, C.C. Johnston Jr., and A.M. Parfitt (eds.), Osteoporosis. Pp. 321-330. Baltimore: University Park Press.

Parfitt, A.M.
1981a The integration of skeletal and mineral homeostasis. In: H.F. DeLuca, H.M.Frost, W.S.S. Jee, C.C. Johnston Jr., and A.M. Parfitt (eds.), Osteoporosis. Pp. 115-126. Baltimore: University Park Press.

Parfitt, A.M.
1983 The physiologic and clinical significance of bone histomorphometric data. In: R.R. Recker (ed.), Bone Histomorphometry: Techniques and Interpretation. Pp. 143-223. Boca Raton, Florida: CRC Press.

Parmalee, P. and W. Klippel
1974 Freshwater mussels as a prehistoric food resource.

American Antiquity 39:421-434.

Parsons, V.
1980 A Color Atlas of Bone Disease. London: Wolfe Medical Publications Ltd.

Pennington, J.A.T. and H.N. Church
1980 Bowes and Churches' Food Values of Portions Commonly Used. Toronto: J.B. Lippincott Company.

Perzigian, A.J.
1973 Osteoporotic bone loss in two prehistoric Indian populations. American Journal of Physical Anthropology 39:87-96.

Pfeiffer, S. and P. King
1983 Cortical bone formation and diet among protohistoric Iroquoians. American Journal of Physical Anthropology 60:23-28.

Pfeiffer, S.
1985 Comparison of adult age estimation techniques, using an ossuary sample. Canadian Review of Physical Anthropology. Pp. 13-17.

Piepenbrink, Hermann
1986 Two examples of biogenous dead bone decomposition and their consequences for taphonomic interpretation. Journal of Archaeological Science 13:417-430.

Plog, F., C,J. Jolly, and D.G. Bates
1980 Anthropology: Decisions, Adaptations, and Evolution. New York: Alfred A. Knopf.

Prentice, A.I.D.
1967 Autofluorescence of bone tissue. Journal of Clinical Pathology 20:717-719.

Price, T.D. and M. Kavanagh
1982 Bone composition and the reconstruction of diet: examples from the Midwestern United States. Midcontinental Journal of Archaeology 7(1): 61-79.

Pugliarello, M.C., F. Vittur, B. de Bernard, E. Bonucci and A. Ascenzi
1973 Analysis of bone composition at the microscopic level. Calcified Tissue Research 12:209-216.

Race, G.J., F. Wendorf, S.B. Humphreys, and E.I. Fry
1972 Palaeopathology of ancient Nubian human bone studied by chemical and electron microscopic methods. Journal of Human Evolution 1: 263-279.

Reed, W.P., D.L. Palmer, R.C. Williams Jr., and A.L. Kisch
1970 Bubonic plague in the Southwestern United States. Medicine 49:465-486.

Richman, E.A., D.J. Ortner, and F.P. Schulter-Ellis
1979 Differences in intracortical bone remodeling in three aboriginal American populations : possible dietary factors. Calcified Tissue International 28:209-214.

Roche, J.
1972 Les amas coquilliers (conchieros) mésolithiques de Muge (Portugal). In: J. Lüning (ed.), Die Anfänge des Neolithikums vom Orient bis Nordeuropa, Teil vll. Westliches Mittlemeergebiet un Britische Inseln. Pp. 72-107. Köln: Böhlau Verlag.

Roche, J and O. da Veiga Ferreira
1972-1973 Sécond datation par le C14 de l´amas coquiller mésolithique de Moita do Sebastião (Muge). Communicações dos Sevicos Geologicos de Portugal LVl:471-474.

Roels, O.A.
1967 Vitamins A and carotene: occurrance in foods. In: W.H. Sebrell Jr., and R.S. Harris (eds.), The Vitamins. Pp. 113-121. New York: Academic Press.

Ruff, C.B. and C.H. Hayes
1982 Subperiosteal expansion and cortical remodeling of the human femur and tibia with aging. Science 217:945-947.

Runyan,T.H.
1976 Nutrition for Today. New York: Harper & Row Publishers.

Sabeau, M.
1985 Personal communication. Department of Endocrinology. University of Alberta.

Saitta, D.J.
1981 Cortical involution of rib in two prehistoric Amerindian populations. In: D.L. Martin and M.P. Bumstead (eds.), Biocultural Adaptation: Comprehensive Approaches to Skeletal Analysis. Pp. 47-63. Research Reports No. 20. Department of Anthropology. University of Massachusetts, Amherst.

Salomon, C.D. and N. Haas
1967 Histological and histochemical observations on undecalcified sections of ancient bone from excavations in Israel. Israel Journal of Medical Science 3:747-754.

Sandison, A.T.
1968 Pathological changes in the skeletons of earlier populations due to acquired disease, and difficulties in their interpretation. In: D.R. Brothwell (ed.), The Skeletal Biology of Earlier Human Populations. Pp. 205-243. Toronto: Pergamon Press.

Saville, P.D.
1965 Changes in bone mass with age and alcoholism. Journal of Bone and Joint Surgery 47:492-499.

Schantz, E.J.
1973 Seafood toxicants. In: E.M. Foster and F.M. Strong (eds.), Toxicants Occurring Naturally in Foods. Pp. 424-447. Washington, D.C.; National Academy of Sciences.

Schoeninger, M.J. and C.S. Peebles
1981 Effect of mollusc eating on human bone strontium levels. Journal of Archaeological Science 8:391-397.

Selye, H.
1956 The Stress of Life. New York: McGraw-Hill Company.

Sigmon, B.A.
1970 Distribution of bone mineral in the femur. In: J.R. Cameron (ed.), Proceedings of the Bone Measurement Conference. Chicago, May 22-23, 1970. Pp. 428-435. Chicago, Illinois: United States Atomic Energy Commission. Division of Technical Information.

Simkiss, K.
1975 Bone and Biomineralization. Great Britain: Camelot Press.

Singh, I.J. and D.L. Gunberg
1970 Estimation of age at death in human males from quantitative histology of bone fragments. American Journal of Physical Anthropology 33:373-382.

Singh, M., A.R. Nagrath, and P.S. Maini
1970 Changes in trabecular pattern of the upper end of the femur as an index of osteoporosis. Journal of Bone and Joint Surgery 52-A:457-467.

Singh, M., L. Riggs, J.W. Beabout, and J. Jowsey
1972 Femoral trabecular pattern index for evaluation of spinal osteoporosis. Annals of Internal Medicine 77:63-67.

Sissons, H.A.
1971 The growth of bone. In: G.H. Bourne (ed.), The Biochemistry and Physiology of Bone. Pp. 145-180. New York: Academic Press.

Smith, R.W. Jr. and R.R. Walker
1964 Femoral expansion in aging women: implications for osteoporosis and fractures. Science 145:156-157.

Smith, R.W. Jr. and B. Frame
1965 Concurrent axial and appendicular osteoporosis. New England Journal of Medicine 273:73-78.

Smith, R.W. Jr. and J. Rizek
1966 Epidemiologic studies of osteoporosis in women of Puerto Rico and southeastern Michigan with special reference to age, race, national origin and to other related or associated findings. Clinical Orthopaedics 45:31-48.

Smith, P.E.L.
1976 Food Production and Its Consequences. Don Mills, Ontario: Cummings Publishing Company.

Souci,S.W., W. Fachmann, and H. Kraut
1981 Food Composition and Nutrition Tables. Stuttgart:Wissenschaftliche mbh.

Spatz, C. and J.O. Johnston
1976 Basic Statistics: Tales of Distribution. Monterey, California: Brooks/Cole Publishing Company.

Spencer, H., L. Kramer, C.A. Gatza, and M. Lender
1979 Calcium loss, calcium absorption, and calcium requirement in osteoporosis. In: U.S. Barzel (ed.), Osteoporosis 11. Pp. 65-89. New York: Grune and Stratton.

Srikantia, S.G.
1982 Vitamin A deficiency and blindness in children. In: A. Neuberger and T.H. Jukes (eds.), Human Nutrition. Pp. 185-196. Englewood, New Jersey: Jack K. Burgess, Inc.

Steinbock, R.T.
1976 Paleopathological Diagnosis and Interpretation. Springfield, Illinois: Charles C. Thomas.

Stini, W.A.
1973 Evolutionary implications of changing nutritional patterns in human populations. American Anthropologist 73(2):1019-1030.

Stini, W.A.
1985 Growth rates and sexual dimorphism in evolutionary perspective. In: R.I. Gilbert and J.H. Mielke (eds.), The Analysis of Prehistoric Diets. Pp. 191-226. Toronto: Academic Press, Inc.

Stothers, D.M. and J.H. Metress
1975 A system for the description and analysis of pathological changes in prehistoric skeletons. Ossa 2:3-9.

Stout, S.D. and S.L. Teitelbaum
1976a Histological analysis of undecalcified thin sections of archaeological bone. American Journal of Physical Anthropology 44:263-270.

Stout, S.D. and S.L. Teitelbaum
1976b Histomorphometric determination of formation rates of archaeological bone. Calcified Tissue Research 21:163-169.

Stout, S.D.
1978 Histological structure and its preservation in ancient bone. Current Anthropology 19(3):601-604.

Stout, S.D. and D.J. Simmons
1979 Use of histology in ancient bone research. Yearbook of Physical Anthropology 22:228-249.

Stout, S.D. and S.J. Gehlert
1980 The relative accuracy and reliability of histological aging methods. Forensic Science International 15:181-190.

Sweeney, E.A. , J. Cabrera, J. Urrutia, and L. Mata
1969 Factors associated with linear hypoplasia of human deciduous incisors. Journal of Dental Research 48:1275-1279.

Tam, C.S., D.R. Swinson, A.H. Little, and B.C. Cruickshank
1976 A biorhythm in osteogenesis. In: Z.F.G. Jaworski (ed.), Proceedings of The First Workshop on Bone Morphometry. Ottawa, March 28-31,1973. Pp. 211-213. Ottawa: University of Ottawa Press.

Teitelbaum, S.L.
1983 Osteoporosis and the bone biopsy. In: L.V. Avioli (ed.), The Osteoporotic Syndrome. Pp. 115-121. New York: Grune and Stratton, Inc.

Thompson, D.D.
1979 The core technique in the determination of age at death in skeletons. Journal of Forensic Sciences 24(4):902-915.

Thompson, D.D.
1980 Age changes in bone mineralization, cortical thickness, and Haversian canal area. Calcified Tissue International 31:5-11.

Thompson, D.D., E.M. Salter, and W.S. Laughlin
1981 Bone core analysis of Baffin Island Skeletons. Arctic Anthropology XVIII-1:87-96.

Thompson, D.D. and Gunness-Hey
1981 Bone mineral-osteon analysis of Yupik-Inupiaq. American Journal of Physical Anthropology 55:1-7.

Thompson, D.D. and K.S.Cowen
1984 Age at death and bone biology of the barrow mummies. Arctic Anthropology 21(1):83-88.

Todd, T.W.
1920 Age changes in the pubic bone. 1: The male White
—— pubis. American Journal of Physical Anthropology 3:285-334.

Trotter, M., G.E. Broman and R.R. Peterson
1960 Densities of bones of White and Negro skeletons. Journal of Bone and Joint Surgery 42 A (1):50-58.

Tutin T.G., V.H. Heywood, N.A. Burgess, D.M.Moore, D.H. Valentine, S.M. Walters, D.A. Webb (eds.)
1980 Flora Europa. New York: Cambridge University Press.

Trotter, M., G.E. Broman and R.R. Peterson
1960 Densities of bones of White and Negro skeletons. Journal of Bone and Joint Surgery 42 A (1):50-58.

Ubelaker, D.H.
1974 Reconstruction of Demographic Profiles From Ossuary Skeletal Samples. Smithonian Contributions to Anthropology No. 18. City of Washington: Smithonian Institution Press.

Van der Merwe, N.J. and J.C. Vogel
1978 ^{13}C content of human collagen as a measure of prehistoric diet in Woodland North America. Nature 276:815-816.

Van der Merwe, N.J., A.C. Roosevelt, and J.C. Vogel
1981 Isotopic evidence for prehistoric subsistence change. Parmana, Venuzuela. Nature 292:536-538.

Van Gerven, D.P., G.J. Armelagos, and M.H. Bartley
1969 Roentgenographic and direct measurement of femoral cortical involution in a prehistoric Mississippian population. American Journal of Physical Anthropology 31:23-38.

Van Gerven, D.P., and G.J. Armelagos
1970 Cortical involution in prehistoric Mississippian femora. Journal of Gerontology 25:20-22.

Van Gerven, D.P.
1973 Thickness and area measurements as parameters of skeletal involution of the humerus, femur, and tibia. Journal of Gerontology 28:40-45.

Vaughn, J.
1981 The Physiology of Bone. New York: Oxford University Press.

Villanueva, A.R.
1976 Methods for preparing and interpreting mineralized sections of bone. In: Z.F.G. Jaworski (ed.), Proceedings of the First Conference on Bone Morphometry. Ottawa, March 28-31, 1973. Pp. 341-353. University of Ottawa Press, Ottawa.

Vilter, R.W.
1967 The effects of ascorbic deficiency in Man. In: W.H. Sebrell and R.S. Harris (eds.), The Vitamins. Pp. 457-485. New York: Academic Press.

Vogel, J.C. and N.J. Van der Merwe
1977 Isotopic evidence for early maize cultivation in New York State. American Antiquity 42:238-242.

Wahner, H.W., B.L. Riggs,and J.W. Beabout
1973 Photon absortion method and Singh Index in the detection of osteoporosis- a comparative study. In:R.B. Mazess (ed.), Proceedings of the International Conference on Bone Mineral Measurement. Chicago, Illinois, October 12-13, 1973. Pp. 225-227. United States Department of Health, Education, and Welfare. DHEW Publication No. (NIH) 75-683. Washington, D.C.: United States Government Printing Office.

Wall,J.C., S.K. Chatterji, and T.W. Jeffrey
1979 Age-related changes in the density and tensile strength of human cortical bone. Calcified Tissue International 27:105-108.

Wallman, I.S. and H.B. Hilton
1962 Teeth pigmented by tetracycline. Lancet 1:827-829.

Weinstein, R.S., D.J.Simmons, C.O. Lovejoy
1981 Ancient bone disease in a Peruvian mummy revealed by quantitative skeletal histomorphometry. American Journal of Physical Anthropology 54:321-326.

Wheater, P.R., H.G. Burkitt, and V.G. Daniels
1979 Functional Histology. New York: Churchill Livingstone.

Whitehouse, R.
1986 Personal communication. Department of Medical

Microbiology and Infectious Diseases, University of Alberta.

Whitney, E.N. and E.M. Nunnelly
1981 Understanding Nutrition. New York: West Publishing Company.

Williams, D.E., R.L. Mason, and B.B. McDonald
1964 Bone density measurements throughout the life cycle of the rat. Journal of Nutrition 84:373-382.

Wilson, E.D., K.H. Fisher, and P.A. Garcia
1979 Principles of Nutrition. Toronto: John Wiley and Sons.

Windle, W.F.
1969 Textbook of Histology. Toronto: McGraw Hill Co.

Wing, E.S. and A.B. Brown
1979 Paleonutrition. Toronto: Academic Press.

Wolbach, J.B.
1947 Vitamin A deficiency and excess in relation to skeletal growth. Journal of Bone and Joint Surgery 29:171-192.

Wood, P.C.
1976 Guide to Shellfish Hygiene. Geneva: World Health Organization, Offset Publication No. 31.